IMPROVEMENT IN LITTER QUALITY AND LEG HEALTH BY NUTRITIONAL MODIFICATION IN GROWING TURKEYS

BY

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Declaration

This thesis has been composed by me and is a record of work carried out in an original line of research, except where due reference is made to the contribution of others. All sources of such information are again listed in the reference section. Also various types of kind help provided during my work is sincerely appreciated by means of appropriate acknowledgements.

None of this work has been presented in any previous application for a degree.

Muhammad Waseem Mirza

Dedication

To my parents, wife and son for being my support and inspiration.

Publications

Part of the work reported in this thesis has been communicated at different conference meetings.

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List of abbreviation

AA:	Amino acids
AAN:	Amino acid nitrogen
ADMO:	Average daily moisture output
ADWI:	Average daily water intake
AME:	Apparent metabolizable energy
AMEn:	Apparent metabolizable energy corrected to nitrogen equilibrium
ANP:	Atrial natriuretic peptide
ATP:	Adenosine triphosphate
BM:	Basal metabolism
BSE:	Bovine spongiform encephalopathy
BW:	Body weight
CP:	Crude protein
CPD:	Coefficient of crude protein digestibility
DC:	Digestibility coefficient
DM:	Dry matter
DMD:	Coefficient of dry matter digestibility
EER:	Energy efficiency ratio
FCE:	Feed conversion efficiency (obtained by dividing body weight gain by food intake)
FI:	Feed intake
FPD:	Footpad dermatitis (interchangeable with footpad score (FPS))
GE:	Gross energy
GIT:	Gastro intestinal tract
GLMM:	Generalised linear mixed model
HB:	Hock burn (interchangeable with hock score (HS))
HI:	Heat increment
LM:	Litter moisture
LS:	Litter score
LSD:	Least significant difference
MO:	Moisture output
ND:	Coefficient of nitrogen digestibility
NDF:	Neutral detergent fibre (neutral detergent fibre intake (NDF I))
NEx:	Nitrogen excretion
NSP:	Non starch polysaccharides
OM:	Organic matter
OMD:	Coefficient of organic matter digestibility

OME:	Organic matter efficiency
OMEx:	Organic matter excretion
OMI:	Organic matter intake
OMR:	Organic matter retention
PER:	Protein efficiency ratio
SED:	Standard error of the differences
SEM:	Standard error of the means
SMR:	Standard metabolic rate
Ta:	Thermoneutral environment
UAN:	Uric acid nitrogen
WI:	Water intake
WG:	Weight gain
WHC:	Water holding capacity

Abstract

Pododermatitis (FPD) is a contact dermatitis commonly observed in poultry, primarily affecting the surface of the footpad and the hock joint, and causes poor welfare and economic losses when severe. Most reported field outbreaks of FPD have been associated with poor litter conditions. There are three important aspects of litter condition associated with incidences of FPD and hock burns (HB) i.e. increased litter moisture, greasy or capped litter as well as high ammonia (NH_3) content. Therefore maintaining litter quality and more specifically the moisture content is essential if conditions such FPD and HB are to be controlled. Poor litter condition is caused by an interaction between management, nutrition and intestinal health. In terms of nutrition, dietary density i.e. energy and protein concentrations are important factors in terms of determining litter quality and incidences of FPD, because of the effect that they exert on water intake.

Four experiments were used to investigate the effects of nutritional modifications on water intake (WI) and excretion by turkeys. In the first experiment explored the effect of different dietary nutrient concentrations supplemented with and without phytase on WI and excretion. It was noted that excreta moisture content was reduced ($P<0.001$) as nutrient density decreased whereas nutrient density had no effect ($P>0.05$) on the cumulative WI. Water output (g/g of weight gain) was higher ($P<0.05$) for phytase-fed birds but nutrient density had no effect ($P>0.05$).

In the next two experiments floor-pen studies were used to examine the effects of nutrient density and dietary protein concentration (ranging from 77 to 120% of BUT breed recommendation) on litter quality parameters and, therefore, on leg health conditions. In one study the energy and protein ratio were kept constant whereas in the second the protein concentration changed while the energy remained constant (100% of breed requirement). Growth performance parameters were determined for each study which was conducted from 4 to 20 weeks of age. When birds were fed diets in which the energy and protein ratio remained constant the high protein/energy diets resulted in a lower WI and litter moisture content when compared to group fed diet containing lower concentrations of protein/energy ($P<0.05$). In contrast litter pH and NH_3 concentration and prevalence of HB were higher when birds were fed with the high protein/energy diets. Notably there was no effect ($P>0.05$) of treatment on FPD.

Birds fed diets containing a higher than the recommended dietary protein concentration (constant energy concentration) had a higher WI and litter moisture content when compared to group fed diets containing the low nutrient density diets ($P<0.001$). Likewise,

litter pH and NH_3 concentration and prevalence of HB and FPD were higher where birds were fed the higher than recommended protein concentration diets.

The final experiment was designed to establish the relative importance of protein and potassium in determining WI and excretion. There were six treatments based on three diets containing either 77, 100 and 120% of the dietary protein recommended by the breeder. Each diet was then split into two and one of the two diets was supplemented with K_2CO_3 to give a K^+ concentration of 16.5 g/kg of diet. The remaining diet of the pair was left unsupplemented (ie contained only naturally occurring potassium). It was noted that birds fed with diet containing higher dietary protein concentration had higher WI and moisture output (MO) when compared to group fed diet containing lower dietary protein concentration ($P<0.001$). The effect within diets containing the same CP and standardised K^+ was marginally insignificant ($P=0.065$) in terms of WI. Whereas birds fed diets containing naturally occurring K^+ only had approximately 10% less ($P<0.05$) MO compared to these fed diets containing the standardised concentration of K^+.

While recognising that factors such as non-starch polysaccharides (NSP), indigestible fat and trypsin inhibitor could not be excluded totally, it was concluded, on the basis of the experiments conducted, that dietary protein (as provided by soybean meal) was primarily responsible for the higher WI and hence excretion. This then ultimately produces unacceptable litter quality and results in leg health problems in turkeys. To prevent excessive water intake and reduce litter moisture content there should be a correct balance between dietary energy and protein levels. Feeding turkeys lower ideal protein diets containing higher apparent metabolisable energy ratio crude protein (AME:CP) may help to improve the amino acid digestibility and ionic balance and, therefore, litter quality and this will help to decrease leg health problems such as footpad dermatitis and hock burn.

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Chapter 1

1 Literature Review

1.1 Introduction

Spaniards brought the turkey (*Meleagris gallopavo*) from North America to Europe in the 16th century from where it was brought in to the United Kingdom (UK) (Feltwell, 1963b). Now, according to FAO (2008), approximately 6.1 million tonnes of turkey meat is produced annually worldwide, of which Europe produces 1.64 million tonnes and the UK 0.14 million tonnes or 4.1 % of the 3.4 million tonnes total meat produced in the UK.

Bovine spongiform encephalopathy (BSE) or 'mad cow disease' is a neurological disorder in animals. It is generally accepted that eating the tissue of animals that have BSE can lead to similar disease in humans (Pattison, 1998). An outbreak of BSE in the UK was linked to the feeding of animals with bone and meat meal. So a ban on the use of animal protein for feeding animals was introduced in some countries. Similarly the use of in-feed antibiotics for animals used for their meat can result in bacteria resistant to antibiotics used in humans, and there was a concern that these bacteria might infect humans feeding on these animals (Phillips, 1999; Ratcliff, 2000). Hence, following increased pressure from consumers and medical groups as well as governments, a European Union (EU) wide ban on the use of most in-feed antibiotics was proposed to be implemented followed by, in 2006, a complete ban on the use of animal by-products in animal feed, under regulation 1831/2003 of the Economic and Social Committee of the European Union (2005).

The ban of in-feed antibiotics in poultry was an important contributor to changes in the intestinal tract microbial ecology of poultry (Dumonceaux *et al.*, 2006), contributing to the emergence of a number of nutrition related problems. One issue that affects chickens, but more often turkeys, is increased levels of moisture in excreta which affects not only bird welfare but also the economic profitability of producers (Martland, 1985).

The ban on the use of animal by-products (except fish meal) in poultry diets in the UK resulted in a reliance on vegetable protein sources, mainly soybean, meal in the diet. There is evidence (Vieira & Lima, 2005) that birds fed with vegetable protein have a higher water intake, possibly because of an imbalance in amino acids and/or ions as well as other factors such as increased non-starch polysaccharides (NSP) (hemicelluloses, pectins and oligosaccharides) content. The dietary factors interact in a complex manner to influence water excretion, but a key dietary factor is the concentration of protein and the amino acid balance. Any osmotic disturbance in the GIT due to higher than normal intake

of proteins, carbohydrates, fats and electrolyte imbalance in poultry diets can results increase water consumption leading to a wet litter problem (Bradshaw *et al*., 2002).

Several studies have reported a correlation between feed composition, faecal viscosity and litter moisture with the prevalence of contact dermatitis (pododermatitis or footpad dermatitis FPD) in turkeys (McIlroy *et al.,* 1987, Bruce *et al.,* 1990; Ekstrand *et al.*, 1997; Ekstrand & Carpenter, 1998; Mayne, 2005). Contact dermatitis may be common under certain conditions and causes poor welfare when severe and, unlike chickens, in turkeys severe pododermatitis is a common lesion (Berg, 1998). Closely related to pododermatitis are “hock burns” (HB). The exact cause of these lesions is unknown but the predisposing factors are complex. Although FPD is a multifactorial problem field outbreaks have tended to be associated with poor litter conditions (Martland, 1984; Green *et al*., 1985) and the water content of the litter (Mayne *et al.*, 2007), a parameter that is directly influenced by the water content of the faeces. It is not surprising therefore, that there have been reports correlating increased water consumption with the incidence of FPD (Manning *et al.*, 2007a). The optimum litter moisture content is somewhere within the range of 25 to 35%, higher litter moisture (LM) is presumed to provide conducive environment which encourage greater microbial degradation of uric acid excreted by the birds into the litter and release more ammonia which exacerbate the problem (Carey *et al*., 2004). Ferguson *et al*. (1998) confirm the relationship between higher litter moisture and increased litter ammonia. Therefore, a change in dietary nutrient levels can alter LM and the production of ammonia by varying the amount of nitrogen available (Carey *et al*., 2004). Conditions that lead to higher moisture in the litter tend to increase ammonia release and high concentrations of ammonia in poultry house. Associated with increased respiratory disease and burning effect of ammonia and other chemical factors from the litter causing FPD and HB (Tucker & Walker, 1992; Gordon & Tucker, 1993). Management of broiler litter to reduce ammonia volatilization is largely a matter of controlling LM and pH therefore, water loss more than normal can create problems in poultry production that include difficulty in maintaining litter quality and associated footpad dermatitis and hock burns (FPD and HB).

Diet density (i.e. energy and protein levels) is considered to be important factors in terms of determining litter quality and incidences of pododermatitis (Bilgili *et al*., 2005; Bilgili *et al*., 2006). High dietary protein when combined with low energy levels may have a more direct effect upon the development of contact dermatitis, by causing uric acid overload in kidneys and thus results in wet capped litter with higher nitrogen concentration (Gordon *et al*., 2003). According to some studies nearly 40% of feed nitrogen in commercial broilers is lost to the atmosphere (Patterson & Lorenz, 1996; Patterson & Lorenz, 1997; Patterson *et al*., 1998). Indeed Nagaraj *et al*. (2007b) reported that high dietary protein level has

been found to associate with the increased incidence and severity of FPD and hock burn in broilers possibly due to the chemical burning effect of high ammonia content in the litter (Bray & Lynn, 1986; Tucker & Walker, 1992). With both economics and animal welfare issues at stake, research aimed at investigating the effect of feed components on litter quality and reducing incidences and severity of contact dermatitis in turkeys is of interest to poultry producers. The purpose of the work reported in this thesis is to assess, identify and to investigate dietary factors with a focus on specific proteinaceous factors that influence water intake and excretion and ultimately influence litter quality.

1.2 **Importance of water in poultry nutrition**

Water is an essential nutrient (Leeson *et al.*, 1976; National Research Council, 1994) and is one of the most abundant – making up about 530-630g/kg of live weight in birds (Larbier & Leclercq, 1994; McDonald *et al.*, 1996). Water is essential for almost all biochemical and physiological body functions (Pfeiffer *et al.*, 1995) such as transporting substances, blood volume maintenance, thermoregulation, cellular homeostasis, digestion and metabolism of nutrients, excretion of waste products and lubrication. Consequently water deprivation can adversely affect production (Adams, 1973) and, while poultry can survive for weeks without feed, they can only last days without water (Barboza *et al.*, 2009).

Despite water being an essential nutrient, its abundance and relatively low price compared to other nutrients make it less attractive to scientists for nutritional studies (Mroz *et al.*, 1995). The knowledge of quantitative requirement of water is more complex because the conventional methods of establishing nutrient requirement cannot be applied directly to water intake (Mroz *et al.*, 1995). The reason for this complexity is the variable amount of water required to meet different physiological function (Schiavon & Emmans, 2000). These complications may have resulted in poor data availability and discrepancies in terms of actual water requirement.

1.3 **Sources of water**

There are three major sources from which birds can obtain water. Each source contributes a variable percentage of a bird's daily water requirement e.g. drinking water (about 76-80%) (Riek *et al.*, 2008), water present in food ingredients (5-15%) and oxidation or metabolic water (about 15%) (Leeson *et al.*, 1976).

1.3.1 Drinking water

Animals drink water primarily to replace lost fluid, rather than in anticipation of future need, and, therefore, drinking water, the most obvious route of water intake, is a thirst motivated process controlled by the stimulation of hypothalamus due to osmoreceptors, mechanoreceptors and rennin-angiotensin axis (Takei, 2000).

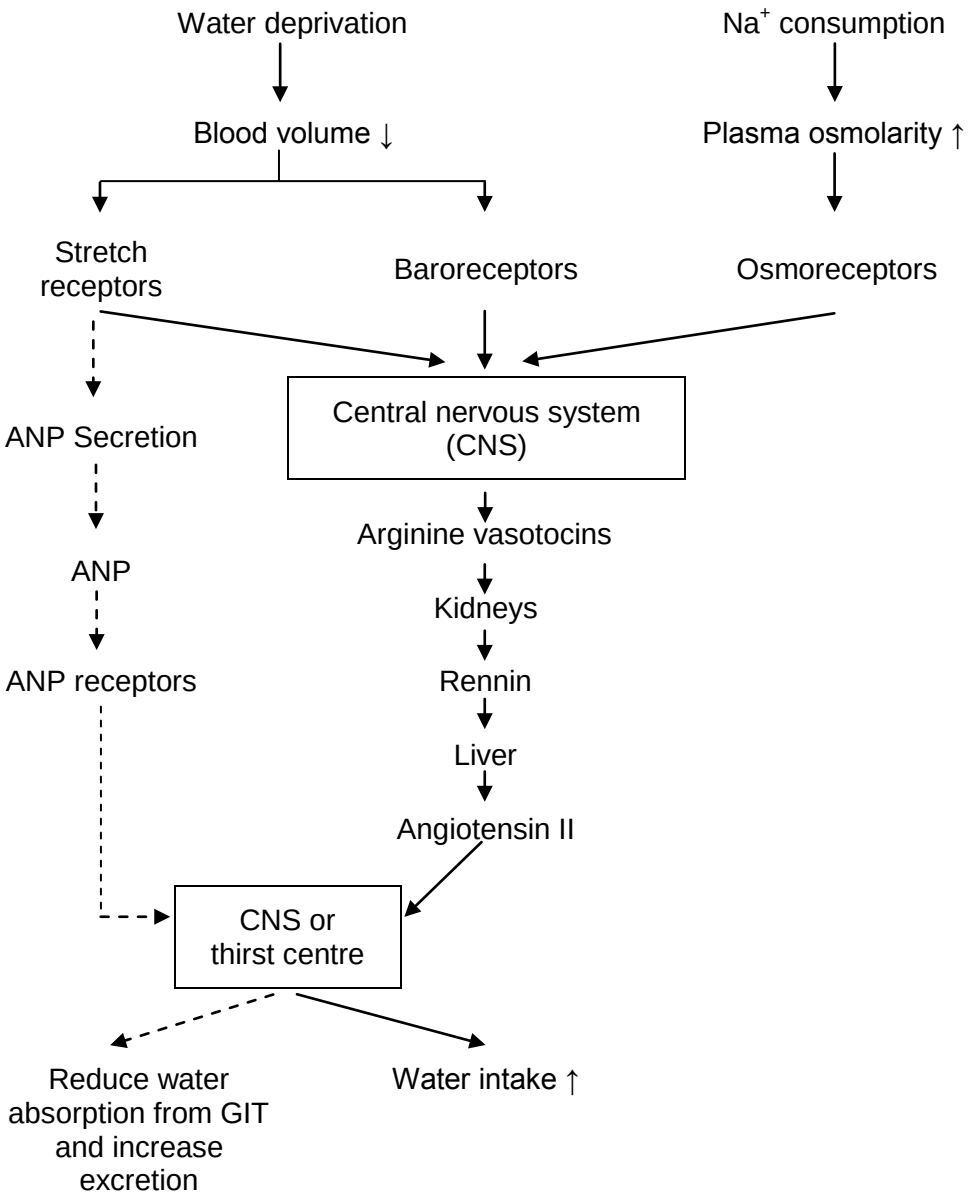


Diagram adapted from (Takei, 2000)

Where,

ANP = Atrial natriuretic peptides

Broken lines = Indicate inhibitory signals

Figure 1: Events that lead to drinking following water deprivation.

The principal stimulation for thirst comes from increased plasma osmolality (Mroz *et al.*, 1995) and the fall in blood volume (Takei, 2000). An increase in the Na^+ concentration in the hypothalamic and juxtaventricular regions due to a higher sodium consumption or following water deprivation and haemorrhage can produce thirst (Larbier & Leclercq, 1994). Angiotensin II a potent dipsogenic hormone is responsible for water intake (Fitzsimons, 1998). Plasma Angiotensin II concentration increases, as the plasma osmolality and Na^+ concentration increases, and blood volume decreases (Takei *et al.*,

1988). According to Figure 1 a water deficit or extracellular dehydration results from a higher plasma osmolality, and factors causing a decrease in blood volume and increase in Angiotensin II. This stimulates the hypothalamic thirst centres responsible for induction of drinking behaviour both directly and through angiotensin II (Takei *et al.*, 1988; Takei, 2000).

Atrial natriuretic peptide (ANP) is a cardiac hormone responsible for antidipsogenic stimuli (Takei, 2000). According to Figure 1 inhibition of ANP secretion due to lower blood volume results in decreased inhibitory signals to the thirst centre through ANP receptors leading to increased water intake.

1.3.2 Water from feed

Typical compounded feed contains on average about 50-150g/kg of structural (or bound) and functional (biologically active) water, of which only the structural water becomes available to the bird during digestion (Leeson *et al.*, 1976). The importance of these water sources varies from animal species and geographical locations. Animals adapted to an arid environment get a significant proportion of their total water requirement from sources other than drinking water as compared to the animals adapted to more humid climate (Church, 1991). For example, the main source of water for kangaroo rats is the preformed water in ingredients and the metabolic water produced within the body (Schmidt-Nielsen, 1972; Schmidt-Nielsen, 1990).

1.3.3 Metabolic water

Metabolic water is a by-product of the metabolism of nutrients (protein, fats and carbohydrates) and depends upon the amount of hydrogen present in the food stuff, hydrogen molecules being required for the formation of metabolic water (Schmidt-Nielsen, 1990). It is generally believed that poultry yields 0.135 g water for the conversion of feed into 1 Kcal - e.g. consumption of 300 Kcal/d yields ≈ 40 g water, which can be used to meet ≈ 15% of daily water requirement. Where glucose yields ≈ 55.5% of its weight, protein yields ≈ 41.5% of its weight and fat yields > 100% of its weight. The oxidation of each gram of protein, fat and carbohydrate can produce about 0.5, 1.2 and 0.6 g of water respectively (Leeson & Summers, 2001).

1molecule of glucose + $O_2 \rightarrow CO_2$ + H_2O+ energy

1molecule of palmitate + $O_2 \rightarrow CO_2$ + H_2O + energy

1g protein + $O_2 \rightarrow CO_2$ +H_2O+ energy + uric acid

Equation 1: Water production as a result of oxidation of glucose, palmitate and protein.

Birds have a relatively high metabolic rate compared with larger animals so the tendency is to produce a greater amount of metabolic water also (Mulkey & Huston, 1967). As indicated by basal metabolism (BM) calculations for mammals and birds (Larbier & Leclercq, 1994; McDonald *et al.*, 1996) large animals have almost a 16% lower BM as compared to birds i.e. BM= 0.3 $W^{0.75}$ and BM= 0.35 $W^{0.75}$ for large mammals and *Gallus* males respectively (where BM is in MJ per day and $W^{0.75}$ is metabolic body weight).

In some animals oxidation of nutrients and even the animal's own tissues e.g. deposited fat makes a net contribution to the total body water pool e.g. camel, fat-tailed sheep, kangaroo rats and even in pigs (Hill, 1976; Skipitaris, 1981). But this contribution of water is not similar when there is higher protein intake. Pfeiffer (1995) while working on pigs, reported that protein metabolism produces a net water deficit. This is probably the result of higher urinary water excretion to dissipate thermal energy produced as a result of protein metabolism. Water loss due to protein metabolism in kangaroo rats is higher than that with lipid and carbohydrate metabolism. Similarly diet composition can affect the water balance (Frank, 1988) and this may also be related to the higher water consumption and excretion when turkeys are fed high protein diets.

1.4 Factors that affect water intake

Factors which can affect water intake in animals are: age, genetics (polydipsia), feed (form, composition and intake), diseases, environmental temperature, watering system and stocking density (Leeson *et al.*, 1976; Obeidah *et al.*, 1977; Marks & Pesti, 1984; Larbier & Leclercq, 1994; Deeb & Cahaner, 2002; Furlan *et al.*, 2004; Riek *et al.*, 2008). The most important factors are highlighted below.

1.4.1 Interaction with feeding

Water intake is closely associated with feed intake. The normal ratio of water to feed reported for poultry is 2:1 and for pigs it can be in the range of 2.5:1 and 5.0:1 (Schiavon & Emmans, 2000). Feed intake stimulates gastric secretions; these gastric secretagogues stimulate sensory receptors which in turn initiate vagus nerve impulses causing hypothalamic stimulation and ultimately water intake (Houpt *et al.*, 1986). Feed intake also

causes dryness of the oropharyngeal receptors, stimulation of stretch receptors and increased gastric mucosal blood flow which then stimulate the hypothalamus to initiate water intake.

In the case of feed restriction an increase in water intake can be caused by an attempt of the bird towards satisfy or simply out of boredom (Mroz *et al.*, 1995; Leeson & Summers, 2000; Viola *et al.*, 2005).

1.4.2 Feed chemical composition

Water intake is mainly affected by the nutrient intake and chemical composition of the diet offered which affects the osmotic pressure within gastro intestinal tract (GIT). The normal average osmotic pressure in the GIT of laying hens ranges from 312-650 milliosmoles (mOsm) (Duke, 1986). Higher nutrient and ion intakes can increase osmotic pressure of the GIT and result in increase water intake.

Water input and output linked to nutrient utilisation in pigs, as reported by Schiavon & Emmans (2000), can provide a basis for determining water demands for each physiological function. The information provided can be related to some of the several steps of the process of nutrient utilisation and on the basis of that water intake (WI) can be predicted as:

$$WI\ (kg/day) = WD + W_{fec} + WE + WG + WU - (WF + WO + WS)$$

Where,
WI = Water intake
WD = Water for digestion
W_{fec} = Water for faecal excretion
WE = Water for evaporation
WG = Water for growth
WU = Water for urine excretion
WF = Water gain from food
WO = Water arising from oxidation of nutrients
WS = Water arising from tissue synthesis

Equation 2: Water requirement for different physiological process.

Schiavon & Emmans (2000) worked on the basis that “the hydrolytic reaction requires 1 mol of water for each mol of simple nutrient released”. This may be applicable for other monogastric animals. The authors suggested that the water requirement for digestion of

carbohydrate, protein and lipids can be calculated according to the following principal e.g. Water for carbohydrate is:

$$WCHO\ (kg/day) = \frac{\text{Molecular mass of water (18)}}{\text{Molecular mass of monosaccharide (162)}} \times \text{Dig. mass of carbohydrate (kg/d)}$$

Where,
WCHO = Water requirement for carbohydrate digestion

Equation 3: Water requirement for digestion of carbohydrate.

The same applies for the calculation of water requirement for the digestion of protein and lipids. Using the average molecular mass of amino acid and fatty acid and assuming them to be the ultimate digestible product of protein and lipid respectively, the calculations of water requirement for the digestion of the dietary constituents can be represented as:

$$WD\ (kg/day) = (18/162)\ DCHO + (18/110)\ DCP + 18/268)\ DL$$

This is equivalent to:

$$WD\ (kg/day) = (0.11)\ DCHO + (0.16)\ DCP + (0.07)\ DL$$

Where,
WD = Water for digestion
18 = Relative molecular mass of water
162 = Relative molecular mass of glucose -1 mol water
110 = Relative molecular mass of amino acid -1 mol water
268 = Relative molecular mass of fatty acid + 1/3 relative mass of glycerol -1 mol water
DCHO = Digestible carbohydrate mass (kg/d)
DCP = Digestible crude protein mass (kg/d)
DL = Digestible lipids mass (kg/d)

Equation 4: Calculations of water requirement for the digestion of the dietary nutrient.

This equation indicates that a reduction in faecal and urinary nutrient excretion can help to reduce water demand for these two physiological process and that this can be achieved by providing a diet made up of highly digestible nutrients. Evaluation of water retention by constituents is also desirable.

1.4.3 Ambient temperature

Birds, like mammals, are warm blooded which means that they control their body temperature. This is done by physical (behavioural) and chemical temperature regulation. In cold environmental temperatures bird huddle together so that they can reduce heat loss and can engage in kleptothermy which literally means 'to steal each others body temperature'. Birds produce heat by transforming nutrients and stored fats into energy. So low temperatures can result in a high energy cost to maintain body metabolism and may require extra feed which can affect water intake. Standard metabolic rate (SMR) is the estimate of energy produced during resting in a thermoneutral environment (Ta) = 22 °C, indicated as average oxygen consumption (Buchholz, 1996; Pekins, 2007). Haroldson (1998) estimated the effect of winter temperature on female wild turkey (0.5-1.5 year old) SMR, found an average SMR of 28.7 $mlO_2 \cdot min^{-1} \cdot bird^{-1}$ by turkeys in a thermoneutral environment (Ta). According to Hill (1976) a 10 °C drop from Ta results in an extra 9ml/min of O_2 consumption requiring the bird to take in another 0.24 MJ/day which could be about 20% more feed in a day. Bearing in mind the water to feed ratio of 2:1 this could significantly increase water intake.

The relevance of this to commercial turkey production is questionable but it demonstrates the link between low temperatures and water intake.

At higher ambient temperatures, bird drink more water and increase water loss in order to adjust their body temperature, either through evaporative heat loss (panting) or directly onto the litter through higher faecal moisture content, in both cases it lead to increased water intake (Collett, 2009). This in turn raises litter moisture content which reduces its friability and overall litter quality (Pattison, 1987). At 20°C water intake is approximately twice that of feed but at 26°C this ratio can rise to 2.5:1 and at 35°C to 5:1 (Collett, 2009).

1.4.4 Dehydration

Dehydration is defined as excessive loss of body water creating a deficiency of fluid within the body. At standard temperature or at cold environmental temperature the most common cause of dehydration in animals is diarrhoea. When body water loss exceeds water intake it cause a reduction in circulatory fluid volume and hydrostatis which then causes an increase in osmotic pressure, so to compensate this depletion extracellular fluid move in to plasma (Leeson & Summers, 2001). Dehydration has been reported to affect young birds the most due to smaller extracellular water pool (Medway & Kare, 1959b), production losses in adult birds and high mortality in turkeys (Ross, 1960;

Marsden *et al.*, 1965). In short, adequate access to water is imperative for optimum performance.

In the case of dehydration, all three regulation mechanisms (explained in Section 1.3.1) for water intake get stimulated resulting in a significant increase in water intake, but the main contributors for increase in water intake are mechanoreceptors and angiotensin II (Takei *et al.*, 1988). Because of sluggish absorption from the intestine and slower cellular rehydration this over stimulation results in higher water intake than actually required by the bird (Takei *et al.*, 1988; Collett, 2006). Slower water absorption from intestine also suppresses plasma arginine vasotosine (antidiuretic hormone in birds) and production of ANP (diuretic hormone) which further reduce water absorption from the intestine and results in higher water excretion (Takei, 2000). Over hydration may also affect normal renal functioning of the bird resulting higher water excretion and wet litter condition (McWhorter *et al.*, 2004).

1.5 ***Water* balance**

Under normal physiological conditions birds, like other animals, have a precise mechanism to maintain homeostasis and constant water level in the body through control on water and ionic composition (Leeson *et al.*, 1976; Schmidt-Nielsen, 1990; Takei, 2000; Collett, 2006; Collett, 2009). This vital control is called water balance and is achieved through maintenance of a dynamic equilibrium between intracellular, interstitial and plasma component of the body fluid (Leeson *et al.*, 1976; McDonald *et al.*, 1996; Leeson & Summers, 1997). Water movement starts when there is disturbance in osmolality between intracellular fluid and plasma. In case of water deficit there will be decrease in blood volume and increase in its osmolality. This will stimulate production of hormone responsible for increase in water intake or conservation from kidneys as reported in Section 1.3.1.

A disturbance in water balance occurs in healthy animals when nutritional stress exceeds the homeostatic capacity and in the case of disease when the functionality of cells responsible for water transport are adversely affected (Collett, 2009). The temperature of the drinking water can influence water intake and, therefore, water balance; the production state of the bird will affect water intake e.g. hens in lay drink more water (Leeson & Summers, 2001).

1.6 **Water output**

Water output or loss in birds can occur by three routes i.e. evaporative losses (from body surface and respiratory organs), through excreta (makes almost 80% water output of which 63% is urinary and 37% is faecal excretion) and in case of laying birds, product (eggs) (Larbier & Leclercq, 1994; Leeson & Summers, 2005; Collett, 2006). Birds have one excretory route for urine and faeces so most of the time higher urinary water output can be wrongly interpreted as the result of diarrhoea or enteritis (Collett, 2006). Urinary output increases with higher excretion of nitrogen and mineral. Whereas faecal water excretion is a direct consequence of dietary factors such as ingesta osmolality, undigestible nutrients, digesta viscosity and by reduction in absorptive function and surface area of intestine (Collett, 2006).

1.6.1 Effect of growth

During the rapid growth phase, birds excrete higher quantity of faecal and urinary water (Collett, 2006). Hermans *et al*. (2006) reported that cases of wet litter were more prevalent in young birds. When comparing growing pullets with adult hens Lopez *et al*. (1973) reported that due to the greater anabolic demand the body water of pullets (proportional to body weight) was greater, about 75.2% compared to adult hens which was 57.8%. The probable reasons for a higher water content could be higher demand for parameters like hematocrit, evaporatory water loss and basal metabolic rate (Medway & Kare, 1959a; Medway & Kare, 1959b). The difference of cockerels and pullets in terms of water intake, could be a combined effect of faster growth rate and higher feed intake in cockerels and, therefore, making their average daily water intake higher then pullets (Chapman & Mihai, 1972; Balogun *et al.*, 1997).

1.6.2 Role of digestive physiology

Some dietary factors such as nutrient imbalances, anti-nutrients, toxins etc., can have a direct or an indirect effect on the normal regulation of water intake and can cause damage to the physiological and normal gut functioning (physiological or ecological) (Lister, 2006) and may result in higher moisture excretion. The effects of these factors can be variable depending upon the amount and nature of undigested feed (Leeson & Summers, 2005). Pfeiffer (1995) reported that water requirement of pigs' increases in case of surplus minerals and nitrogen.

A well developed GIT improves the efficiency of nutrient utilisation (Moreto & Planas, 1989; Swatson *et al.*, 2002). GIT renewal and ability to transport nutrient depends upon age and dietary factors, mainly energy and protein (Soguero & Vinardell, 1994). Digestibility of lysine and arginine can be reduced due to higher dietary NaCl content (Chen *et al.*, 2005) resulting in amino acid imbalance, affecting growth, crypt depth and villus height, fat metabolism and ionic permeability as well as lower enzyme activity in the GIT (Swatson *et al.*, 2002). Aldosterone hormone is essential for the protein synthesis in the intestinal wall (Soguero & Vinardell, 1994) and production of this hormone reduces due to higher dietary NaCl, so poor GIT development can effect digestion and absorption of nutrients and can be reason of higher faecal moisture excretion.

A single dose of water labelled with isotopes diffuses from the digestive tract and into blood within 90 minutes in ducks as compared to 180 minutes in reindeer and seals (Barboza *et al.*, 2009). This shows that the short GIT length in birds requires a rapid absorption of water and anything which can hinder this may cause watery faeces.

1.6.3 Role of temperature

High environmental temperatures can increase the amount of evaporative water loss from the respiratory tract (Mulkey & Huston, 1967). It can be estimated by amount of water loss per unit of oxygen consumption (Hill, 1976) and depends mainly on respiratory physiology of the species whereas, within species it depends upon factors such as temperature and humidity (Larbier & Leclercq, 1994). Values reported for birds at moderate temperature and low humidity range from 0.6 mg/ml O_2 to 3.7 mg/ml O_2 (Hill, 1976). In extreme cases respiratory evaporation can approximate water intake so poultry houses need good ventilation to avoid excessive moisture built up (Leeson & Summers, 2005).

1.7 **Impact of moisture on the litter and the bird**

1.7.1 Litter material

Litter quality is one of the most important components in floor rearing system, especially for broilers and meat producing turkeys as these birds stay in contact with litter throughout their life (Nagaraj *et al*., 2007c; Lister, 2009). A good quality litter should satisfy the bird's welfare requirements by absorbing moisture, providing a warm and dry surface to rest on, to provide a substrate that allows microbial activity to degrade faeces, and to encourage dust bathing and scratching. As a guideline, if a hand full of tightly squeezed litter upon opening the fist crevices and falls easily without forming a cohesive ball and has around

75% dry matter content, it will be regarded as dry litter (North & Bell, 1990). Although the precise range of moisture content is provided in the Section 1.7.2, the choice of commonly used litter materials e.g. wood shaving, clean chaffed wheat straw and peat moss depends largely on availability and economics (Feltwell, 1963b). Generally, wood shaving is preferred over wheat straw as it is more absorbent and can release moisture easily, bird can turn wood shaving more easily (Meluzzi *et al*., 2008a). Whereas, wheat straw develops a strong crust which prevents moisture release and gas emission and, therefore, can increase the risk of FPD (Bruce *et al*., 1990; Shanawany, 1992; Benabdeljelil & Ayachi, 1996; Ekstrand *et al*., 1997; Lien *et al*., 1998; Su *et al*., 2000; Dozier *et al*., 2005). The most important characteristics of litter quality are; it should be clean, absolutely dry, dust free, homogeneous, lightweight, good insulation properties, disease free, non toxic and should not be mouldy (Feltwell, 1963a; Brake *et al*., 1992; Lister, 2009).

1.7.2 Wet litter

Wetness of the poultry litter is a direct consequence of water addition (urine/faecal/spillage) in excess to the rate of removal (evaporation) (Collett, 2006). The term wet litter is used when bedding material reaches saturation threshold and cannot hold more moisture (Hermans *et al.*, 2006) and loses its friability (Pattison, 1987). According to Lister (2009) the normal critical value for litter moisture content is 250g/kg of litter and beyond that it should be considered “wet”. Others have said that moisture contents exceeding 350g/kg are more likely to produce health problems (Collett, 2007; Collett, 2009; Lister, 2009). Higher litter moisture can create health, welfare (Mayne *et al.*, 2007), management and higher NH_3 production problems (Carr *et al.*, 1990; Nahm, 2007).

Although little information is available on the prevalence of wet litter, results of a comprehensive survey done by Hermans & Morgan (2007) indicates that wet litter is a common phenomenon in UK commercial broiler farms. It has been reported by Hermans *et al*. (2006) that about 57% farm managers who responded to a questionnaire indicated wet litter condition in the last broiler flock they have reared. Despite the higher risks involved with wet litter in poultry production it is often not fully appreciated (Ritz *et al.*, 2005).

1.7.3 Causes and consequences of wet litter

There are a number of factors which cause wet litter condition in poultry, mainly divided in to infectious (diseases) and non infectious categories (Lister, 2009) whereas, further subdivisions of non infectious category include feed composition/form, season, age of the

bird, sex, genetics, drinker design and number, litter depth, stocking density, weather and ventilation. Figure 2 shows the likely causes of poor litter quality or wet litter condition, which can lead to the condition of hock burn and footpad damage. Bird behaviour can also be affected by nutrition, however, not been quantified in term its effect on of water usage.

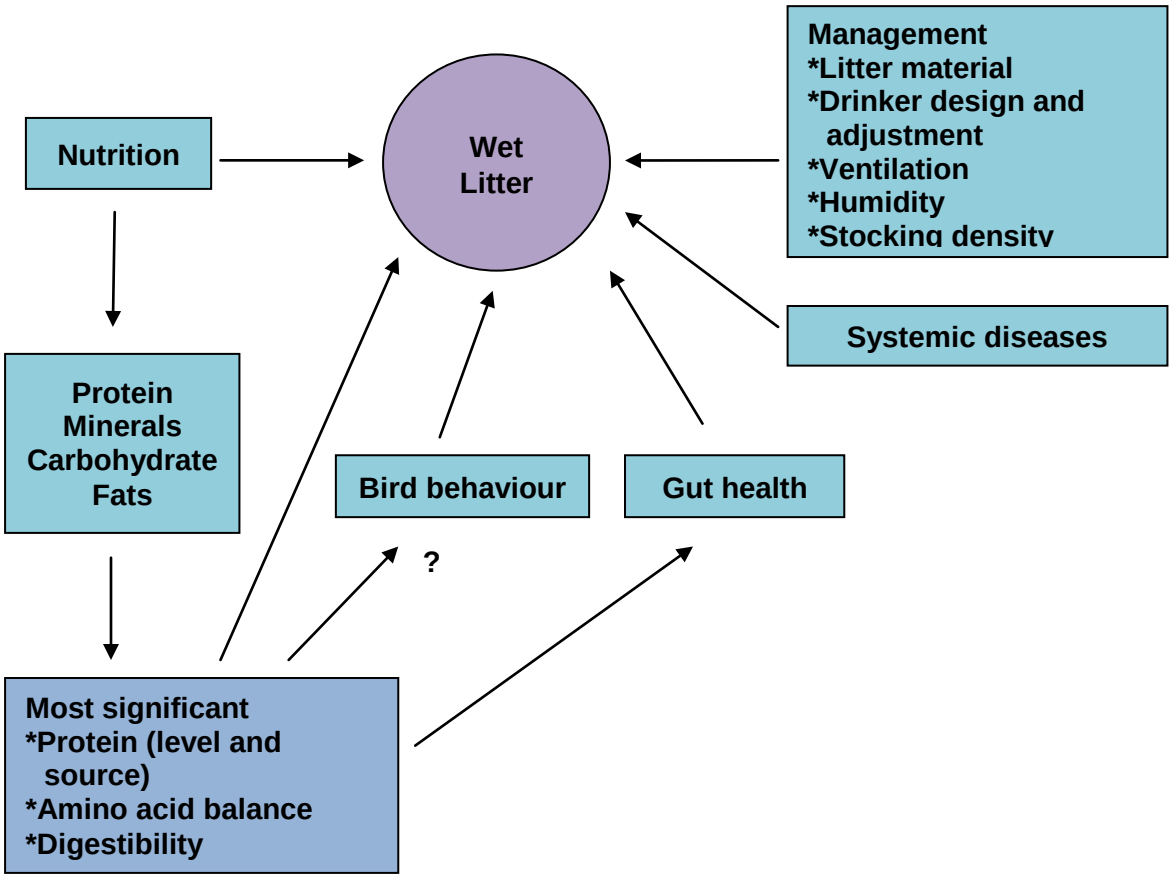


Figure 2: Reasons of wet litter condition in poultry.

Some of the problems associated with wet litter are highlighted in the following sections.

1.7.3.1 **Ammonia and litter**

Ammonia (NH_3) is a colourless, irritant gas with sharp and penetrating odour produced from nitrogenous compounds of animal waste by microbial activity (Carlile, 1984). There are different factors involved in the production of NH_3 in the poultry house (Liu *et al.*, 2007) but a higher NH_3 concentration is mainly associated with higher litter moisture and nitrogen contents (Elwinger & Svensson, 1996; Shah *et al.*, 2007). It has been reported as one of the most harmful gases (Liu *et al.*, 2007) and a smallest concentration can cause stress (Dawkins *et al.*, 2004) and health problems in poultry (Homidan *et al.*, 2003; Nicholson *et al.*, 2004; Shah *et al.*, 2007). Toxic effects of ammonia at many different levels were highlighted by Cooper & Plum (1987) in a review. NH_3 and, high litter moisture

content are correlated with dirty footpads, FPD and hock burn (HB) lesions in poultry (Ekstrand *et al.*, 1997; Dawkins *et al.*, 2004; Haslam *et al.*, 2006; Mayne *et al.*, 2007).

The UK has agreed to reduce its yearly NH_3 production (DEFRA, 2001). The estimated total NH_3 emission in year 2000 was 320 kilo tonnes of which about 80% contribution was from agriculture sources with 17% of agricultural contribution coming from poultry houses (DEFRA, 2001). NH_3 is also a major environmental nuisance due to its bad smell (Shah *et al.*, 2007). The control on NH_3 production in commercial poultry is possible through a modification in nutritional and management practices (Dawkins *et al.*, 2004; Nicholson *et al.*, 2004; Pratt *et al.*, 2004).

1.7.3.2 **Manure management**

Plant-based bedding material along with chicken excrement, feathers, and spilled feed are the principal components of litter, providing a dynamic component of poultry house environment (Fuller *et al*., 2004). Most broiler operations produce 1.1 to 1.07 tons of litter per 1,000 birds (Patterson *et al*., 1998) however, Chamblee & Todd (2002) estimated broiler litter production to be 1.6 tons per 1,000 broilers if the houses were cleaned out completely on an annual basis, and a rate of 1 ton per 1,000 broilers if houses were cleaned out completely at the end of 2nd year. Proper management of litter in the poultry house will reduce the need to remove litter between flocks and will aid in developing a cleanout schedule that allows direct application of manure to cropland without intermediate storage.

In avoiding water or environmental pollution due to poultry manure, higher litter moisture is often regarded as one of the most important limiting factors. Higher moisture contents also make handling and management of poultry manure difficult. This can be costly especially if one has to dry it (Smith *et al.*, 2000b; Shaw *et al.*, 2006). There are certain alternative ways for manure handling. Electricity generation by burning poultry used litter is already in practice (Smith, 1996) but there are upper acceptable limits on the water content (Smith, 1974; Henuk & Dingle, 2003).

1.7.3.3 **Birds health and disease prevalence**

High litter moisture can affect economic returns of commercial poultry production. Losses can occur due to breast burns, FPD, HB and scabby areas, bruising, condemnation and downgrades, respiratory diseases, higher pathogenic load as well production of dirty eggs (Ekstrand *et al.*, 1997; Smith *et al.*, 2000b; Kjaer *et al.*, 2006; Mayne *et al.*, 2007). There

are a number of other associated problems such as the proliferation of flies which can also serve as a vector of many diseases (Murakami *et al.*, 2003). Flies are carrier of some of most dangerous diseases of poultry such as avian influenza, colibacillosis, laryngotracheitis, gangrenous dermatitis, gumboro, diseases caused by retrovirus, bronchitis and botulism (McMullin, 1998; Ritz *et al.*, 2005). Higher litter moisture also increases the chances of toxic fungi proliferation, which may cause mycotoxicosis. Higher number of beetles and mites can be produced as a result of higher litter moisture which besides being irritating also cause structural damage. Damage to structures including insulating material, fibre glass and wooden frames can lead to higher energy cost to keep the temperature constant in the house. High litter moisture is the ultimate reason for high pH (Carr *et al.*, 1990) in the litter by changing it from 5.2 to 8.2. This rise in pH along with humidity and temperature creates an ideal environment for uric acid splitting bacteria to produce NH_3 (Pattison, 1987). Furthermore higher litter moisture content also increases the risk of coccidiosis, worm infestation such as tapeworms and round worms may be by providing conducive environment for their survival (DEFRA, 2000).

1.8 **Factors affecting leg health**

Genetic selection of meat producing birds to increase muscle mass quickly and as efficiently as possible has led to an increase in metabolic diseases including, more specifically, leg health conditions (Knowles *et al*., 2008; AHAW, 2010; Ask, 2010). So birds can exhibit a range of skeletal and locomotor problems caused by infectious and non-infectious leg disorders (Mench, 2004), resulting in poor walking ability, or locomotion being a primary concern (Julian *et al*., 1986; Broom, 1987; Broom, 1993; Norci & Montella, 2003; Havenstein *et al*., 2007). Apart from the obvious welfare implications these disorders can have a major economic a impact as birds with such disorders may have difficulty accessing feed and water, while the increased tonic mobility, increased fear response leading difficulty reaching the food and water, may be trampled by other birds, and may experience pain (Sanotra *et al*., 2001a; Campo *et al*., 2005).

The aetiology of leg disorders is complex but includes genetics, nutrition, age, growth rate, sanitation, lighting, litter quality, stocking density and other environmental and management factors (Gordon & Tucker, 1997; Dawkins *et al*., 2004; Mench, 2004). These categories are not mutually exclusive as one aetiology factor may affect another. However, disorders may be classified according to underlying pathology as infectious or non-infectious (developmental and degenerative).

Infectious disorders causing leg problems include arthritis/tenosynovitis, infectious stunting syndrome, viral induced neoplasia, and bacterial chondronecrosis with osteomyelitis (BCO) (sometimes called ‘femoral head necrosis’ or ‘proximal femoral head

degeneration'). Developmental conditions include varus valgus disease (VVD), rotated tibia, tibial dyschondroplasia (TD), rickets, chondrodystrophy and spondylolisthesis. Degenerative disorders include osteochondrosis, epiphyseolosis, degenerative joint disease, spontaneous rupture of the gastrocnemius tendon and contact dermatitis. It is difficult to assess all disorders in relation to frequency of occurrence and their impact on welfare due to a lack of published data. However it is estimated that leg problems are responsible for 1.1% of broiler mortality and 2.1% of carcass condemnations and downgrades annually, and cost the poultry industry billions of dollars each year (Morris, 1993). BCO, TD, contact dermatitis and VVD disease are believed to be the most important and common leg disorders (Bradshaw *et al*., 2002).

1.9 Causes and prevalence of infectious disorders

Leg disorders caused by infectious agents (e.g. bacteria and viruses) are largely responsible for severe lameness (e.g. BCO) while those caused by illness of a non-infectious origin cause less severe lameness (e.g. TD) (Lynch *et al*., 1992; Bradshaw *et al*., 2002). The complexity of the aetiology of these causes has resulted in contradictory reports in the literature however one of the most important infectious disorders of the leg is BCO. Surveys of commercial flocks in Scandinavia (Sanotra *et al*., 2001b; Sanotra & Berg, 2003) showed an incidence of BCO in Swedish flocks ranged from 0 to 24% (average 10.4%) of the total recorded skeletal disorders.

1.10 Causes and prevalence of non-infectious disorders

The causes of non-infectious leg disorders in meat-type poultry are generally believed to be linked to rapid growth (Sorensen, 1992). Leg disorders of non-infectious aetiology are more common to occur as compared to infectious origin (Bradshaw *et al*., 2002). One of the most important developmental conditions, TD, is considered a heritable disorder that can cause lameness. Recorded mean incidences of TD and VVD in Denmark and Swedish flocks were believed to be around 57.1 (ranging from 32 to nearly 90%) and 37.0% (ranged from 5 to 74%) respectively, of the total recorded skeletal disorders (Sanotra *et al*., 2001b; Sanotra & Berg, 2003).

Although contact dermatitis (FPD and HB) was previously considered to be an environmental problem (McIlroy *et al*., 1987) recent reports (Ask, 2010) have suggested that it can be controlled through genetic selection (as a long term strategy). In the immediate term however there is a considerable amount of literature that relates FPD with litter quality and more specifically the moisture content of litter (Greene *et al*., 1985; Martland, 1985). As the moisture content of litter is closely linked to feed and hence water

intake (Pond *et al*., 1995; Fuller *et al*., 2004) it is the aim of this research programme to identify the key nutritional factors that predispose to litter conditions that in turn can cause pododermatitis.

1.10.1 Pododermatitis (footpad dermatitis, FPD) and hock burn (HB)) causes, Impact

- **Prevalence of FPD and HB**

Among the various leg abnormalities, commercially one of the most important problems in turkey production is pododermatitis or footpad dermatitis (FPD) (Ekstrand *et al*., 1997; Mayne *et al*., 2007), which is more prevalent in turkeys than chickens (Berg, 1998). This is in contrast to the HB, the prevalence of which is lower in turkeys than chickens, possibly due to the active nature of turkeys (Pattison, 1987). It has been reported by Ekstrand *et al*. (1997) that when 32% of the commercial broiler flocks in Sweden were studied 10% of the flocks studied were found to be were suffering with FPD. Current UK chicken production standards allow up to 15% of broilers to have HB whereas some data suggest that the figure of affected broilers is around 82% (Broom & Reefmann, 2005). Another more recent study in the United Kingdom found a mean FPD prevalence of 14.8% based on 86 flocks from 21 conventional farms, and a mean FPD prevalence of 98.1% was found based on 128 flocks from 23 organic farms (Pagazaurtundua & Warriss, 2006). Of all reported cases of FPD in the UK the number for turkey males were almost double than females (Clark *et al.*, 2002).

- **Description of FPD**

FPD is a type of contact dermatitis affecting the plantar region of the bird's feet (Meluzzi *et al*., 2008b) and is defined as inflammation of the footpad (Kjaer *et al*., 2006). It is also associated with the hock joint and in severe cases may extend to the breast area (Greene *et al*., 1985). The early signs are associated with discolouration, hyperkeratinisation, thickening and cracking of the footpad skin (Whitehead & Bannister, 1981) affecting both metatarsal and digital pads. This leads to oedema, necrosis of the epidermis (Ekstrand *et al*., 1997), presence of erosive superficial and/or deep lesions which then lead to severe ulceration and bleeding. Crusts, formed by exudates, litter and faecal material often cover the ulcerations (Meluzzi *et al*., 2008b). In turkeys, it would be commonly termed "footpad burns" or "ammonia burns" (Clark *et al*., 2002).

FPD can cause pain resulting in an unsteady walk and the lesions provide potential routes of entry for bacteria (Ekstrand *et al*., 1997). The lesions are often covered by crusts

formed by exudate, litter and faecal material. Irritation from faeces or litter causes a thickening of the foot-pad epidermis (acanthosis and hyperkeratosis). If faeces stick to the foot it may cause ischemic necrosis and ulceration that is accompanied by suffering and pain (Julian & Gazdzinski, 1999; Buda *et al*., 2002). Although not primarily caused by any particular microbial agent, the lesions often become infected and can be a gateway for a variety of bacteria and fungi (Greene *et al*., 1985), especially *Staphylococcus spp.* (Hester, 1994). This microbial penetration may subsequently lead to synovitis and lameness and can cause of impairment of carcass quality in turkeys (Schmidt & Lüders, 1976; Blair, 1978; Martland, 1984; Bowers & Shane, 1997; Berg, 2004).

Closely related to FPD are “hock burns” (HB), in which the skin of the hock becomes dark brown (Kjaer *et al*., 2006), and are likely to cause pain, as a result of tissue trauma, the degree of which will vary with lesion severity (Nairn & Watson, 1972; Harms *et al*., 1977; Greene *et al*., 1985; McIlroy *et al*., 1987; Schulze Kersting, 1996, Berg, 2004).

- **Economic impact of FPD**

According to Haslam *et al*. (2006) only HB lesions are currently measured and recorded in the UK which means that the economic impact of FPD cannot be assessed accurately. The FPD scoring system takes into account only certain stages of developed FPD and information on susceptibility and early stages of FPD is rare (Mayne *et al.*, 2006). Supermarkets in the UK are however conscious of the welfare issues affecting the bird and FPD has been recognised as a potential key indicator for welfare assessment measures (Clark *et al.*, 2002; Haslam *et al.*, 2006). According to (Pattison, 1987) carcass rejection due to HB lesions can result in a loss of 3 to 6 pence per kg.

As a result of consumer’s increased awareness of animal welfare, food quality and environmental protection the consumer and retailer tend to be more critical about the assessment of commercial poultry welfare (Meluzzi *et al*., 2008b). In Europe it has been suggested that producers should be subjected to a penalty if the incidence of FPD is not reduced.

Poultry feet are not used for human consumption in Europe, however, they are regarded as valued food stuff in some parts of the world e.g. Hong Kong (Eichner *et al*., 2007) where poultry feet import was worth $75 million in the first half of year 2006 (USDA-FAS, 2007) making a total worth of poultry feet export market in USA around $280 million (US Poultry & Egg Export Council, 2009). FPD can be the most common reason for downgrading feet during processing and, therefore, it is unacceptable both in terms of welfare and profitability (Menzies *et al*., 1998).

1.10.1.1 **Non-nutritional predisposing or risk factors**

A multitude of factors can predispose to FPD however the most significant are wet litter (Mayne *et al*., 2007) while a high ammonia content, so-called 'ammonia burns' (Tucker & Walker, 1992; Gordon & Tucker, 1993) are also important.

The frequency and severity of lesions on the foot-pads, hocks and breast increase with the age of the birds (Greene *et al*., 1985; Hemminga & Vertommen, 1985; Martland, 1985; McIlroy *et al*., 1987). Mayne *et al*. (2006) found that externally normal foot pads showed microscopic evidence of lesions after the turkeys reached 4 weeks and from 6 weeks of age onwards prevalence and severity of lesions increase as the age progresses and Breuer (2005) reported that young turkey poults might be sensitive to FPD than older one. Ekstrand *et al*. (1997) observed healing of the FPD lesions at an older age provided that birds were fed on less nutrient intense diets.

It has been reported that as body weight increases so there is a decrease in activity, which increases their tendency to spend long periods (McIlroy *et al*., 1987) in close contact with the litter. Therefore, several authors have reported that heavier birds showed a higher incidence of dermatitis (Harms & Simpson, 1975; Hemminga & Vertommen, 1985). Rapid weight gain results in more pressure per area of foot increasing the contact of sensitive areas of the skin to the irritants in the litter produced from fecal load in the litter (Stephenson *et al*., 1960; McIlory *et al*., 1987; Menzies *et al*., 1998).

Contact dermatitis have been exacerbated by genetic selection for fast growth and increased feed conversion (AHAW, 2010) and under experimental rearing conditions the prevalence and severity of FPD can be explained by variance in the genetic lines of turkey and broiler, particularly in those lines with a heavy body weight (Ekstrand *et al*., 1998; Kestin & Sorenson, 1999; Hafez *et al*., 2004, Bilgili *et al*., 2006). Others have not been able to identify any biologically significant differences between different commercially available hybrids under commercial conditions (Ekstrand *et al*., 1997; Ekstrand & Carpenter, 1998). There is a possibility that these differences were a result of difference in environmental and management practices adopted between the two arrangements i.e. experimental vs. Commercial.

The impact of gender is a subject of controversial debate. While some studies showed no difference between hens and toms in the incidence and severity of foot pad dermatitis (Martland, 1984; Ekstrand *et al*., 1997; Berg, 1998), other authors found a higher incidence of foot pad lesions in male birds compared to females (Stephenson *et al*., 1960; Harms & Simpson, 1975; Bruce *et al*., 1990; Cravener *et al*., 1992; Ekstrand *et al* 1997;

Menzies *et al*., 1998; Bilgili *et al*., 2006). However as with age, findings related to gender are confounded to a certain extent by body weight (Berg, 1998; Clark *et al*., 2002).

A recent review of the scientific literature has concluded that stocking density is a central issue for chicken welfare (Bessei 2006). As there is considerable evidence that high stocking density can increase the incidence and severity of leg disorders, contact dermatitis and carcass bruising (Hall, 2001). Rearing broilers at high stocking rates of <0.48 sq ft/bird have been shown to increase leg problems (Grashorn & Kutritz 1991) and pathologies such as chronic dermatitis, leg disorders, while walking ability and general activity are reduced (Hall, 2001). It also leads to a rapid deterioration of litter quality (Mcllroy *et al*., 1987; Bruce *et al*., 1990; Gordon, 1992) and the generation of corrosive or irritant factors due to the high concentration of faeces present in the litter (Martrencher *et al*., 2002). A higher stocking density also leads to poor air circulation in the house and inferior air quality which increases the chances litter deterioration.

A study by Dawkins *et al*. (2004) highlights though that that although very high stocking densities affect broiler welfare, it is not stocking density per se that is important but the environmental conditions (albeit sticking density can impact on these). So factors such as house size and age, litter moisture, air ammonia, temperature, humidity, ventilation and season play an important role in the aetiology of FPD.

1.10.1.2 **Nutritional predisposing or risk factors**

Feed is a “matrix” which forms by the combination of different substances that differ in physical and chemical composition and their interaction (Robertson, 1988). The properties of this matrix affect its digestibility, rate of passage, rate of nutrient availability or transfer from the feed or food to the animal tissues. Feed is the most costly item in commercial poultry production, around 70-80% of the total cost of production (Acamovic, 2001). Any process or factor which results in poor efficiency can reduce the economic output by affecting birds and increasing the volume of animal waste.

Poor quality feed ingredients along with their minerals or excessive oligosaccharide contents can produce nutritionally induced polydipsia and increases nutrient through flow which results in wet litter condition (Collett, 2006). Some details are provided in Section 1.10.1.3.5. The management of bird performance and gut ecology became a challenge after the ban on in-feed antibiotics requiring alternative non antibiotic techniques. This approach consists of an understanding of digestive physiology of the modern poultry and a diet formulation with best nutrient balance close enough to meet the requirement of maintenance, growth and production (Hermans & Morgan, 2007). Feed management factors such as addition of enzymes, mycotoxin binders, prebiotics, probiotics, dietary

dilutions, reduction in anti-nutritional factors, phase feedings, minimizing wastage, attempt to avoid water spillage and precision feeding can minimize wet litter in birds (Collett, 2006).

1.10.1.3 **Relationship of water intake and nutrients**

Certain dietary constituents can have an adverse effect on litter quality, either by causing an increase in water intake which leads to wetter faeces, or by making the faeces sticky. In a number of reports a correlation between feed composition, faecal viscosity and litter moisture with the prevalence of contact dermatitis has been reported (McIlroy *et al*., 1987, Bruce *et al*., 1990; Ekstrand & Carpenter, 1998a; Ekstrand & Carpenter, 1998).

The role of dietary minerals, fat, carbohydrates and diet density as a risk factor for the deterioration of litter quality and hence FPD and HB have been discussed in details in commencing sections.

1.10.1.3.1 Protein

Proteins or more precisely amino acids are one of the most expensive and important components of poultry feed (Moore *et al.*, 2001; Faria Filho *et al.*, 2005; Kamran *et al.*, 2008c) since a bird's protein requirement is actually its amino acid requirement (Firman & Boling, 1998). Due to the direct effect on cost and performance meeting the dietary requirement can be challenging, especially in turkey feeding where the requirement of protein and amino acid is high (Lemme *et al.*, 2004). Comparatively less work has been done to establish the amino acid requirement of turkeys (Firman & Boling, 1998) putting a further constraint on diet formulation (Lemme *et al.*, 2004). Due to a lack of underpinning knowledge, nutritionists tend to favour higher protein contents in the diet to achieve optimum growth (Baker *et al.*, 2003; Kamran *et al.*, 2008b). These practices can result in increased economic pressure and an increase in welfare problems, especially when the diet is poorly balanced (Swatson *et al.*, 2002), which can result in poor digestive efficiency (Nahm, 2007). So for sustainable poultry production there is a need for a critical review of the dietary protein levels required for turkeys (Carr *et al.*, 1990; Blair *et al.*, 1999; Schutte & Dejong, 2004; Si *et al.*, 2004; Waldroup *et al.*, 2005b).

Genetic advancement in growth parameters of the poultry have been achieved at the cost of a reduced retention time in the proventiculus and gizzard (Sklan & Hurwitz, 1980; Collett, 2006). Inefficient utilisation increases the intact protein/peptides through flow especially the soluble ones, due to their faster flow rate. These peptides can contribute to

the osmotic pressure (Leeson & Summers, 1997) in the GIT which in turn can reduce water absorption in the body (Guilford, 1994).

A factor which can increase water intake with higher protein intake is the relatively high heat increment of protein retention 0.036MJ/g as compare to 0.004MJ/g of fat retention (Emmans, 1994). Protein metabolism produces a net water deficit in kangaroo rats as indicated by Frank (1988). Poor energy utilisation from protein metabolism (Musharaf & Latshaw, 1999) may also increase the net water deficit. Frank (1988) reported that each gram of protein produces 0.34g urea which requires 1.458g of water to void it in kangaroo rats.

Birds fed vegetable protein diets had a higher water intake per kg of diet, almost 190g more than birds fed a diet containing poultry by-products and excreted a higher volume of excreta with higher moisture contents (Vieira & Lima, 2005; Vieira *et al.*, 2006). The reasons for the high moisture excretion associated with soybean meal are described in Section 1.10.1.3.5. Soybean is the best alternative to animal protein sources and commonly used ingredient in animal production (Fischer *et al.*, 2007) but an inclusion of more than 20% can cause wet litter (Pattison, 1987). A decrease in water intake by lowering soybean contents in the feed has been reported by Furlan *et al*. (2004).

Despite the importance of dietary protein quality and adequate supply (Faria Filho *et al.*, 2005) an excessive intake requires an increase in water intake (Shaw *et al.*, 2006; Ziaei *et al.*, 2007) to allow the excessive nitrogen to be excreted (Francesch & Brufau, 2004). The strong correlation between dietary protein, nitrogen excretion and litter moisture is supported by a number of studies (Marks & Pesti, 1984; Pfeiffer *et al.*, 1995; Alleman & Leclercq, 1997; Ferguson *et al.*, 1998; Clark *et al.*, 2002; Furlan *et al.*, 2004; Rezaei *et al.*, 2004; Ziaei *et al.*, 2007). Each 10g/kg increase in dietary crude protein (CP) intake increases water intake by 30g/kg of diet (Larbier & Leclercq, 1994) consequently increasing litter moisture, pH and NH_3 (Ferguson *et al.*, 1998). Similar findings were reported by Elwinger & Svensson (1996) working on broiler and by Jirjis *et al*. (1997) while working on turkeys, they found that increases in dietary protein content increases urinary volume and NH_3 emission. However, although wet litter can result from high dietary protein levels there is only limited information available on the quantitative effect of protein on water intake and excretion.

Most of the proteins contain from 300 to 5000 amino acids (Leeson & Summers, 1997) bonded together and the hydrolytic break down of each bond requires a molecule of water and this water has to come from drinking water. Utilisation of peptides depends upon the hydrophobic nature of the peptides. Hydrophilic peptides are poorly absorbed and utilised

by tissues due to poor mucosal hydrolysis, which may also be related to dietary vegetable protein source (Daniel *et al.*, 1992; Pan *et al.*, 1996).

There is considerable interest in the quantitative aspects of efficiency of utilisation of the protein source and the balance of amino acids (Wu, 2009) by poultry. Using balanced diets for domestic fowl, efficiencies in utilisation of dietary protein in the region of 60 to 70% are generally achieved (Scott *et al*., 1982). Higher dietary protein levels can lead to reduction in their utilisation (Summers *et al.*, 1964; Marks & Pesti, 1984). But lowering dietary CP levels without addressing the amino acid profile can result in compromised production performance in poultry (Kamran *et al.*, 2008b). This could be due to an imbalance of essential and non-essential amino acids supply which can affect their utilisation (Waldroup *et al.*, 1976; Ferguson *et al.*, 1998; Heger *et al.*, 1998; Schutte & Dejong, 2004; Corzo *et al.*, 2005). A more efficient utilisation of nitrogen can be achieved in a diet that contains only essential amino acids by adding other sources of non-essential nitrogen. This supports the view that an imbalance in the proportions of amino acids (essential and non essential amino acids) also leads to reduced efficiency and therefore excesses of essential amino acids are less efficiently utilised in poultry (Stevens, 2004). But with the increased usage of synthetic amino acids in poultry feed it is possible to reduce dietary intact protein levels (Thompson *et al.*, 2004; Faria Filho *et al.*, 2005; Thompson & Firman, 2005) without affecting the balance between essential and non-essential amino acids.

Ideal proteins are based on digestible amino acids and can be defined as the exact balance of amino acids within the protein supply that is needed for maximum growth (Firman & Boling, 1998). Formulating turkey diets on an ideal protein basis is believed to be the best way to reduce to optimize the CP content (80 to 100g/kg of the diet) for the welfare of the birds and to resolve environmental problems (Firman, 2004; Lemme *et al.*, 2004; Thompson *et al.*, 2004). Church (1991) puts it as, “if absorbed lysine is in short supply but is required for the protein being synthesised, the amount of synthesis will be governed by the available lysine”, i.e. other essential amino acids, over and above the amount that can be used with lysine, will then be used primarily for energy production rather than functioning as amino acids, resulting in poor protein utilization and increased nitrogen excretion by the birds. Therefore, the balance of amino acids is critical and, once achieved, it provides a reliable and flexible way to meet the requirement for growth and maintenance, and reduction in nitrogen losses (Parsons, 1996; Heger *et al.*, 1998; Firman, 2004). No ill effects on the growth performance of turkeys (Emmert & Baker, 1997; Firman & Boling, 1998) or broilers (Schutte & Dejong, 2004; Kamran *et al.*, 2008a; Kamran *et al.*, 2008b) were reported when fed on the basis of an ideal protein.

No work has been done to date to incorporate any pollution related factors while designing ideal protein for turkeys (Emmert & Baker, 1997). The quantitative effect of an ideal protein on water intake and excretion has not been explored either. There is a need for a data base which can provide information regarding the digestible amino acid requirement of turkeys for all stages of growth (Baker *et al.*, 2003; Koch, 2005) to avoid any oversupply. There might be an over estimation of ideal protein ratios already in practice due to difference of sex, strain and efficiency of amino acid utilisation by different stages of turkeys growth. Chen *et al*. (2005) reported that higher environmental temperature can result differential amino acid digestibilities of the feed ingredients and, therefore, affects their nutritional specification. Hence any over feeding of amino acids can increase nitrogen losses and, therefore, may also increase water excretion in poultry.

When there is an excess of dietary protein, it cannot be stored as such and becomes degraded and deaminated, providing carbon skeletons for biosynthesis of fats and carbohydrates. The surplus nitrogen is excreted. Unlike in mammals, the principal form of nitrogen excreted by birds is uric acid. However, birds excrete nitrogen in all three forms i.e. uric acid, urea and ammonia. The amount of nitrogen excretion varies with dietary protein concentration, whereas the proportional composition of these nitrogenous waste products varies according to the physiological requirement of the bird along with the availability of drinking water. Elevated blood ammonia ion concentration has been shown to alter carbohydrate and fat metabolism and adenosine triphosphate (ATP) levels, not only in the brain, but in other tissues as well (Wiechetek *et al.*, 1979). Furthermore, being toxic, ammonia is excreted with the larger amount of water as compared to the previous two (Sabat *et al.*, 2004). The urea cycle, which produces urea from ammonia, is incomplete in birds due to the absence of carbamylphosphate synthetase (Griminger & Scanes, 1986). Which means that if birds have flexibility in their pattern of nitrogenous waste excretion and have water available *ad libitum* (Tsahar *et al.*, 2005) it is likely to be a variation in the proportions of urates (uric acid bound with cations) and ammonia rather than varying urea excretion (Roxburgh & Pinshow, 2002). O'Dell *et al*. (1960) reported that the sources and level of dietary protein can influence the distribution of urinary nitrogen between uric acid and ammonia. Although the underlying mechanism of the correlation between water intake with ammonotely remains obscure (Aldea & Sabat, 2007), ammonia is osmotically active and toxic and, therefore, requires a significant amount of water to detoxify and excrete it (Mcnabb *et al.*, 1972; Wright, 1995) details are given in Figure 3. Bacterial break down of uric acid in the hind gut (Tsahar *et al.*, 2005) decreases the urate concentration by about 9%, increasing ammonia and urea concentrations by 104 and 97% respectively in the excreted fluid (Roxburgh & Pinshow, 2002), which again require water. As ammonia is toxic, it requires almost 400 ml of water to detoxify 1 g of ammonia (Wright, 1995). Roxburg & Pinshow (2002) noted that

ammonotely can occur in species in which breakdown of urate in the hindgut allow uric acid nitrogen concentrations to fall below ammonia nitrogen concentrations. Higher uric acid excretions also need protein to prevent accumulation in renal tubules (Janes & Braun, 1997). According to Namtound *et al*. (2008) reduction in CP content of broiler diet from 230g/kg to 190g/kg can decrease uric acid and moisture excretion without any ill effects on performance.

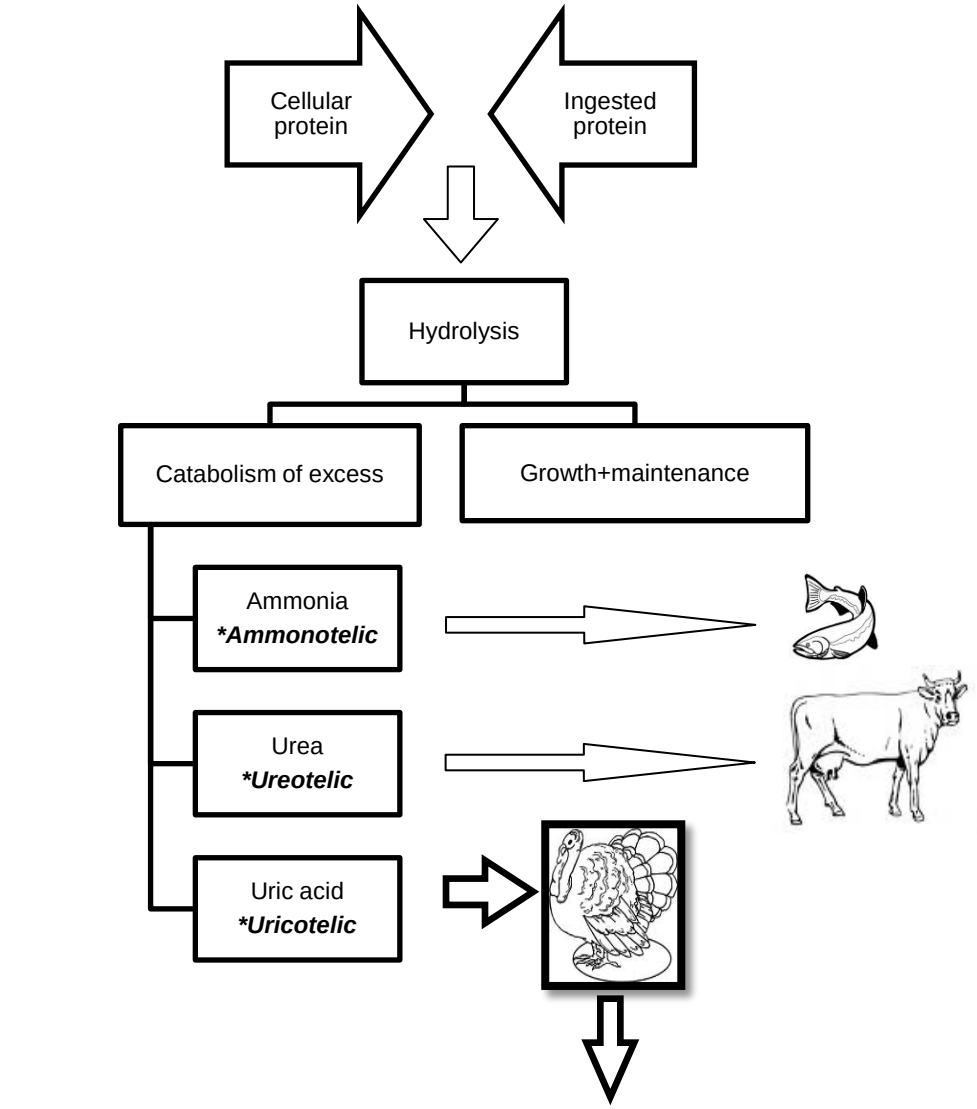
The excretory system of fowl has an additional function of nutrient conservation (osmotic regulation, nitrogen homeostasis, glucose, water and sodium). After a selective reabsorption of nutrients from the kidney, the bird manages to excrete concentrated urine which contains total nitrogen of around 400-450mg/100ml of urine, chiefly consisting of uric acid (Qureshi, 1998). Uric acid is synthesised in the liver of the chicken and excreted through the kidneys, it is insoluble in water and its concentration makes the urine somewhat pasty. In the case of higher uric acid excretion the glomerular filtration rate (GFR) becomes unable to excrete and tubular excretion becomes the main route (Sturkie, 1986). Uric acid makes little or no contribution to the osmolality of the urine due to its characteristic insolubility though it can hold electrolytes, which might have some effect. The tubular section is meant to reabsorb water and any disturbance can impair this normal function resulting in increased urinary water loss. Higher dietary calcium and protein can create problems by modulating renal morphology e.g. enlargement of kidneys and deposition of urates (Leeson & Summers, 2005). Nitrogenous excretion increases linearly with the increase in protein intake and this excretion puts a significant cost of energy to the kidney, therefore, requiring physiological adjustments by a change in renal structure. Though not confirmed by studies on healthy humans, this might causes a progressive loss in renal capacity as a result of renal hypertrophy or increased glomerular filtration rate (Martin *et al.*, 2005). Sabat *et al*. (2004) performed a trial on the omnivorous Rufous-collared sparrow (*Zonotrichia capensis*) and observed a medullary tissues hypertrophy in kidneys of group fed higher protein diet which could be a response of higher amount of nitrogen waste. While working on house sparrows (*Passer domesticus*) Goldstein *et al*. (2001) found that feeding high protein diets increased urine flow to almost double when comparing diets with 80g and 300 g CP/kg. They also observed larger renal medullae in sparrows fed diet with higher protein level with no effect on kidney mass.

It is widely believed that the excretion of nitrogen in the form of urate enables birds to conserve water by excreting semi-solid urine. However, it has been calculated that the formation and excretion of uric acid by the domestic fowl would entail the use of 200 ml water per gram of nitrogen, whereas the excretion of urea by mammals could use 150 ml per gram of nitrogen (King & McLelland, 1984). This indicates that higher dietary protein concentration and a resulting higher uric acid excretion is a significant contributor to water

demand. However Goldstein & Skadhauge (2000) highlighted that birds receiving a low protein diet when had limited energy available (e.g. starving) can have relatively higher quantity of nitrogen excreted in forms other than uric acid it is just to conserve energy. These forms e.g. urea and ammonia are osmotically active and require a lot of water to be excreted. When the dietary energy is lower than what the animal requires they tend to compensate this by increasing amino acid oxidation to use as energy source and that can result in higher nitrogen excretion (Church, 1991; Pfeiffer, 1995).

High dietary protein levels may have a more direct effect upon the development of contact dermatitis, by causing uric acid overload in kidneys and thus results in wet capped litter with higher nitrogen concentration (Gordon *et al*., 2003). The optimum litter moisture content is somewhere within the range of 25 to 35%, higher litter moisture is presumed to provide an environment which is conducive to microbial uric acid degradation, releasing ammonia which exacerbate the problem. Therefore, changes in dietary nutrient levels can alter the production of ammonia by varying the amount of nitrogen available (Carey *et al*., 2004).

Biotin is the vitamin co-factor for pyruvate carboxylase, which forms oxaloacetate, and for acetyl-CoA carboxylase, which is the first step in fatty acid biosynthesis. A high dietary protein level negatively affects the availability and concentration of plasma biotin l (Clark *et al*., 2002) perhaps as a result of increases in nitrogen excretion or enzyme turnover rates (Whitehead & Bannister, 1981), therefore, disrupting the biotin dependent lipogenic pathway involving acetyl-CoA carboxylase which then results in abnormal skin lipid composition and poor skin integrity. Whitehead & Bannister (1981) explained that a high-protein diet requires disposal of excess amino acids, some of which (e.g. alanine) may be metabolised to glucose, for which pyruvate carboxylase is necessary. Under these conditions the enzyme is maintained at a higher relative activity, even at the expense of a small decrease in the amount of biotin available for acetyl-CoA carboxylase. Poor skin integrity results in weak resistance against sticky faeces and micro-organisms (Whitehead & Bannister, 1981; Clark *et al*., 2002; Nagaraj *et al*., 2007a; Nagaraj *et al*., 2007b). Higher litter moisture content might increase the rate of irritants released from the litter and sticky litter probably brings these irritants in permanent contact with the skin (Wang *et al*., 1998).



Proportion of total Nitrogen excreted as **Uric acid**	Proportion of total Nitrogen excretd as **Urea**	Proportion of total Nitrogen excretd as **Ammonia**
• 75-80% • **1g excretion require 8 ml water** • Not toxic (can build up until precipitation) • Require 5 ATP/molecule excreted	• 10-15% • **1g excretion require 40 ml water** • Not toxic (can build up) • Require 2 ATP/molecule excreted	• 5-15% • **1g excretion require 400 ml water** • Toxic (must stay low) • Require almost no energy for excretion

Diagram adapted from (Wright, 1995)

*Three main categories according to the chief nitrogen excretory products. Animals which excrete urea, uric acid or ammonia as chief nitrogenous waste excretion are classified in to the category of *ureotelic*, *uricotelic* and *ammonotelic*, respectively.

Figure 3: General overview of nitrogen metabolism and excretion in animals.

1.10.1.3.2 Fat

Fat (synonym for lipid) due to its higher energy contents is suitable for the production of cost effective and nutritionally efficient animal feed. It also has some additional benefits like improving feed palatability and digestibility, lowering feed dustiness and nutrient segregation and improving vitamin utilisation (Wiseman *et al.*, 1986; Doreau & Chilliard, 1997; Baião & Lara, 2005). Fat can reduce the digesta passage rate which can result in improved dietary nutrient absorption from GIT (Krogdahl, 1985; Baião & Lara, 2005).

There are several factors which can affect fat digestibility and, therefore, can reduce the digestibility of other nutrients (Pattison, 1987). Young chicks are not able to digest fats with higher proportion of saturated fatty acids due to a lack of bile salt production necessary for digestion (Krogdahl, 1985; Pattison, 1987; Wiseman & Salvador, 1989; Doreau & Chilliard, 1997). Fat composition, source, quality and levels can influence overall fat utilisation because different components can be digested with varying efficiency (Zelenka *et al.*, 2003).

Increased carbonic chain length of the saturated fatty acids reduces their solubility in water and increases the melting point causing a significant reduction in utilisation by poultry (Renner & Hill, 1961b; Doreau & Chilliard, 1997). The greater the number of unsaturated fatty acids (e.g. polyunsaturated) increases their solubility and reduces the melting point (Baião & Lara, 2005) which can increase their availability to the birds. Composition of fatty acids can effect the fat utilisation as unsaturated fatty acids can have a synergistic effect on saturated fatty acids by promoting their utilisation by the birds (Renner & Hill, 1961a; Krogdahl, 1985; Wiseman & Lessire, 1987). A higher than 1.5 ratio of unsaturated fatty acids with saturated fatty acids can increase digestibility in non ruminants (Doreau & Chilliard, 1997). Krogdahl (1985) has explained this phenomenon as the micelles formed by mixing of unsaturated fatty acids with bile (forming insoluble amphiphiles) act as liquid crystals to solubilise the long chain saturated fatty acids (non swelling amphiphiles).

Birds fed diet containing fat from animal source (saturated fatty acids) have higher water intake as compared to the ones that have vegetable fat (unsaturated fatty acids) source in their diets and it can lead to the higher litter moisture contents. The relative better utilisations of lipids of vegetable origin as compared to animal fat for broiler chickens were observed by scientists e.g. (Danicke *et al.*, 1999; Mossab *et al.*, 2000; Preston *et al.*, 2001). Whereas increasing dietary fat, to more than 90g/kg of diet, can mask the effects of other nutrients e.g. carbohydrates in the GIT and can reduce their digestibility (Pattison, 1987; Doreau & Chilliard, 1997; Hetland *et al.*, 2004). Therefore high dietary fat levels,

and especially poor quality fat (low digestibility) resulted in excess excretion of faecal fat with droppings have sticky consistency (Bray & Lynn, 1986; Pattison, 1987). These faeces in turn adhere to the footpad of the bird and can cap the litter surface therefore, reduction in litter porosity and inhibiting any moisture movement (Pattison, 1987). Similarly oxidative rancidity occurring due to the oxidation of double bond in unsaturated fatty acids results in reduced digestibility, disturbance in GIT functioning and tissue damage and lastly wet litter condition (Collett, 2006). Since nutrient utilisation depends on integrity of the GIT lining and status of the gut environment (Collett, 2006) this damage to the lining can affect the digestion and absorption and may produce wet litter condition.

1.10.1.3.3 Carbohydrate

Carbohydrates are the back bone of poultry feed formulations and consist of a mixture of polymers that are associated with other non-carbohydrate components (Jozefiak *et al.*, 2004). Carbohydrates are divided into simple and complex carbohydrates and complex carbohydrates can be further divided into starch and non-starch polysaccharides (NSP).

Starch is the concentrated source of energy storage in its native roughly spherical semi-crystalline form in plants (Tester *et al.*, 2004a; Svihus *et al.*, 2005) and is also rated as the main source of energy in poultry feed (Weurding *et al.*, 2001; Carre, 2004; Jozefiak *et al.*, 2004). According to Topping (2007), starch is the most important polysaccharide in nearly all seeds (including legumes). And it makes almost 40-50% of the total poultry feed dry weight (400 to 550g/kg) (Knudsen *et al.*, 2006). So any variation in starch digestibility can affect the energy value of poultry diet (Carre, 2004) and can also effect water intake in poultry. According to Lee *et al*. (2004) feeding rye instead of maize can significantly increase water consumption in broiler chickens due to the anti-nutritional effects on NSP. Johansson *et al*. (1948) reported that the type of dietary carbohydrate has a marked effect on intestinal microflora in hens. There are many factors that can reduce starch digestibility, increase digesta osmolality and excreta moisture content. The next paragraphs will cover the most important aspects which can affect starch digestibility and so affect the amount of undigested material reaching the lower part of the digestive tract.

The chemical composition and structure of starch is mainly dependent on physiochemical properties, compositional variation and molecular interaction of the starch (Tester *et al.*, 2004b). A brief summary of the characteristics which can affect starch digestibility is presented followed by some details. These characteristics are amylose/amylopectin ratio, proportion of A/B starch granules, shape and crystallinity of the starch granules, nature of protein and lipid matrix surrounding them, and overall architecture of the starch granules (Gutierrez-Alamo *et al.*, 2008).

Starch is a very complex structure and depending on its source differences can exist in the nature of the starch and its chemical composition which may influence its digestibility (Tester *et al.*, 2004b). Physical characteristics like granule surface area, starch structure and degree of crystallinity can have impact on its digestibility (Weurding *et al.*, 2001). These factors have further subdivisions according to the botanical source of starch (Robertson, 1988). Starch from wheat or peas show greater variation in digestibility as compared to maize (Carre, 2004). Type of starch is another source of variation in its digestibility and according to Tester *et al*. (2004a) is the predominant regulator of controlling susceptibility to hydrolytic enzymes and on the basis of its types can be divided in three groups A, B and C. Where A is present in cereals, B is part of tubers and C is present in legumes (Weurding *et al.*, 2001). The basis of this division is the presence of high density of hydrogen bonds at certain places of starch molecule which defines the crystalline zones which is partly dependent on the amylose proportion of the starch molecule (Carre, 2004).

Shape of starch molecule can be either round, lenticular or polygonal (Tester *et al.*, 2004a), where lenticular shaped starch may have lower digestibility. Size of starch molecule normally ranges from (~1-100 µm in diameter) (Tester *et al.*, 2004a), and according to Svihus *et al*. (2005) size of starch molecule may affect the digestibility, where small sized starch molecules are reported to have better enzyme substrate relationship and thus have high digestibility. Size distribution of starch molecules that is either uni- or bi-modal, suggests that more the variation in size of starch molecules greater the variation in digestibility (Svihus *et al.*, 2005).

The ratio of amylose to amylopectin may have some effect on starch digestibility and starch granules with higher amylose contents (>40g/100g) tend to be more resistant than others (Carre, 2004). It could be due to a complex formation of lipids with high amylose contents which makes it resistant to water swelling (Weurding *et al.*, 2001; Svihus *et al.*, 2005). Starches with higher amylose contents appeared to be resistant to gelatinisation during feed processing which is an important aspect of reduction in crystalline structure and increasing the chances of amylopectin degradation (Tester *et al.*, 2004a). But according to Pirgozliev *et al*. (2010) the nutritional significance of ratio variation in amylose and amylopectin contents of starch is not clear.

Other factors that affect carbohydrate digestibility are fat and protein covering the starch molecules as they are mainly hydrophobic in nature therefore, can impair the digestibility directly in two ways by reducing the contact of digestive enzyme and indirectly by reduction in the swelling characteristic (Svihus *et al.*, 2005; Knudsen *et al.*, 2006).

Some cereals also contain a considerable proportion of NSP as principal component of their fibre (Carre, 2004; Svihus *et al.*, 2005; Topping, 2007). On the basis of their aqueous solubility, NSP are divided into two categories soluble and insoluble. There is less contribution of soluble NSP towards faecal mass (Topping, 2007) but this increases the bulk of digesta and makes the bird produce sticky droppings (Hetland *et al.*, 2004). They are known to possess anti-nutritional properties by encapsulating nutrients and play a major roll in digesta transit time and viscosity and associated higher water holding capacity (WHC) (Williams *et al.*, 1997; Carre, 2004; Jozefiak *et al.*, 2004). Higher digesta viscosity can hinder the interaction of enzyme substrate and reduce the transport of hydrolytic products across the epithelium of the GIT (Robertson, 1988; Carre, 2004; Jozefiak *et al.*, 2004; Tester *et al.*, 2004a) thus affecting other nutrient's digestibility. The extent of lower digestibility was lowest for starch and maximum for lipids (Carre, 2004). Lee *et al*. (2004) have reported that feeding rye instead of maize impaired fat digestibility by 7.2% units due to higher viscosity in GIT as a result of rye feeding. A study by Van Leeuwen & Jansman (2007) reported that dietary NSP stimulate digesta passage rate through GIT especially in the large intestine of the pigs. The authors also emphasised that higher viscosity and WHC of the ingredient resulted increased digesta mass with delayed transit time from the small intestine and increased transit time of digesta from the last part of GIT. It can also result in higher nutrient loss due to lesser digestion and absorption from the small intestine and might reduce the water reabsorption in the large intestine of the bird. As reported by Van der Klis *et al*. (1995) viscosity have negative relationship with absorption of dry matter and minerals in broilers therefore, according to Williams *et al*. (1997) NSP presence resulted in greater moisture level in the manure.

Schutte *et al*. (1991) reported that when a comparison was made between two groups of pigs, one fed glucose and other xylose, the latter group had significantly higher water intake, urine output and produced faeces with lower dry matter contents. Similar findings were reported when glucose fed group was compared with arabinose fed group where the latter group had a significantly higher water intake and urine output (Schutte *et al.*, 1992a). In a study on broilers Schutte *et al*. (1992b) reported almost the same findings where groups fed xylose and arabinose had a significantly higher water consumption and higher litter moisture content. The utilisation of CP tends to decrease when either xylose or arabinose was included in the diet. This was the result of osmotic properties of unabsorbed pentose sugars and increased volatile fatty acids concentration as a result of bacterial action on these sugars. These studies indicated that higher concentration of undigested sugars can increase the flow of water in to GIT and as a result of that higher excretion in the faeces.

Lastly the unprocessed starch digestibility is different from the processed one (Robertson, 1988; Tester *et al.*, 2004a) as birds do not have any teeth for mastication so particle size and other feed processing are very much linked with starch digestibility. Therefore, prediction of feed digestibility only on its chemical basis may not be true. Other factors, such as feed source, processing procedures and digestive characteristics of the particular species, might have greater influence on digestibility (Robertson, 1988). Weurding *et al*. (2001) reported that animal related factors like age, feed intake, and passage as well as absorption capacity can affect starch digestibility. It has been documented by Knudsen *et al*. (2006) that physical processing (cracking, grinding, roller milling, pelleting, expanding and extrusion) of wheat starch is important to facilitate the water penetration and to make it accessible for α-amylase activity. The effects of feed processing on starch digestibility can be seen in Section 1.10.1.3.9, of this document covering feed processing and its effects on digestibility in poultry.

1.10.1.3.4 Minerals

Sodium (Na^+), potassium (K^+) and chloride (Cl^-) ions are the principal electrolytes in a poultry diet (Roland & Caldwell, 1985; Borges *et al.*, 2007) and are important for body functions like the maintenance of osmotic pressure, acid base balance, nerve signals transmission, optimum growth and bone development (Murakami *et al.*, 2001; Murakami *et al.*, 2003). Beside the minimum required level, the ratio of these dietary electrolytes is critical and has to be maintained (Borgatti *et al.*, 2004). There is a well known relationship between electrolyte balance, environmental temperature and water intake, and excretion (Vankampen, 1981). Though there is evidence that excessive concentrations of dietary minerals can increase excreta moisture in poultry, there is little information available to help quantitatively describe this increase.

Na^+ and Cl^- are combined as NaCl for poultry usage (Cohen *et al.*, 1972), and it has been argued that its low cost is the reason that optimum levels have failed to be established (Murakami *et al.*, 2001). The higher intake of these principal electrolytes causes significant osmotic changes in the intestinal lumen of the bird and can increase water retention in the digesta (Appleby *et al*., 1992; Tucker & Walker, 1992; Murakami *et al*., 2000). Several authors (Mongin, 1981; Wages *et al.*, 1995; Hooge *et al.*, 1999; Smith *et al.*, 2000a; Smith *et al.*, 2000b; Murakami *et al.*, 2001; Oviedo-Rondon *et al.*, 2001; Maiorka *et al.*, 2004; Ahmad & Sarwar, 2006; Borges *et al.*, 2007; Manning *et al.*, 2007a; Manning *et al.*, 2007b) indicated a linear effect of principal electrolyte on water intake and excretion in poultry.

This higher Na^+ excretion, by necessity, means a loss of an equivalent anion (Cl^-) and water (Collett, 2006). However there are some contradictory reports about the effect of Cl^- on water intake and excretion (Oviedo-Rondon *et al.*, 2001; Murakami *et al.*, 2003). Shaw *et al*. (2006) have reported that excess minerals did not affect the average daily water intake but that they did increase water excretion through faeces in pigs. Hawthorne & Markwell (2004) tried two different levels of Na^+ in trial with cats and concluded that Na^+ was correlated with the water intake and urinary output in cats. Any excess of excretory Na^+ can induce renal hypertrophy reducing the functionality of the kidney to reabsorb water (Larbier & Leclercq, 1994).

Smith *et al*. (2000a) have reported in a study on laying hens that for a 1g increase in sodium, potassium and phosphorus in per kg of the feed from normal levels there was an increase of 9.04, 11.95 and 5.59ml of water excretion respectively. According to Smith *et al*. (2000b) these electrolytes in combination with other fractions in the diet like beta glucans can cause production of sticky excreta with higher moisture contents. These sticky faeces can be collected on the footpads of the birds causing irritation, which subsequently may induce FPD (Martrenchar *et al.*, 2002; Mayne, 2005).

Soybean meal contains relatively high amounts of K^+ (Collett, 2006) which is the most abundant intracellular cation (Borges *et al.*, 2007) and has a tendency to increase the urinary water loss and litter moisture content – the result of higher levels of K^+ and a lower dry matter digestibility.

The interaction of minerals with each other is an important factor in animal nutrition but interaction at different sites i.e. at site of absorption, transport and metabolism increase the complexity of this relationship (Leeson & Summers, 1997). Although there are reports which confirm that an interaction of Na^+ with K^+ can lead to higher moisture level in poultry excreta and could cause a wet litter problem (Smith *et al.*, 2000a). But according to Ahmad *et al*. (2005) the determination of the optimum dietary mineral concentration is difficult because of the interaction between them as well as environmental effects on feed consumption and metabolism.

Calcium (Ca^{++}) and phosphorus (P^+) are often come together in discussions and excess of one can precipitate the other in the intestine. Excess Ca^{++} can increase the calcitonin level (diuretic hormone) and can cause urolithiasis which results in reduced renal ability to retain water therefore potentially causing wet litter. An excess of Ca^{++} and P^+ in the diet can interfere with the absorption of manganese. The alkaline environment in duodenum facilitates excessive calcium and phosphorus to reform in a flocculent precipitate of calcium phosphate. This absorbs manganese and zinc and washed them out of intestine

(Leeson & Summers, 1997). Magnesium can also cause diuresis and wet litter (Leeson & Summers, 2005).

1.10.1.3.5 Other dietary factors

Some dietary ingredients, when fed in excess, increase the intake and subsequent excretion of water. These ingredients have been classified as viscous grain (Choct, 2006) due to their ability to increase viscosity in GIT and water intake. Legumes such as soybean are one of most commonly used vegetable protein sources in poultry feed (Kocher *et al.*, 2002), and are believed to contain more complex NSP than cereals (Leeson & Summers, 2005; Broz & Ward, 2007) and to have higher levels of indigestible fats (Mayne, 2005).

There are reports that feeding high levels of soybean meal as main protein source can cause sticky and high pH droppings with high moisture content resulting in wet and litter that contains irritants (Abbott *et al*., 1969; Jensen *et al*., 1970; Nairn & Watson, 1972; Whitehead & Bannister, 1981; Jensen, 1985). The indigestible oligosaccharides component of the soybean meal has been implicated as a factor in causing sticky and potentially irritant droppings and wet litter conditions (Jensen *et al*., 1970; Boling & Firman, 1997; Bilgili *et al*., 2005). Soybean meal has a naturally higher K^+ content (Bradshaw *et al*., 2002), trypsin-inhibitor and NSP contents when compared to other vegetable protein sources which increase water consumption leading to a watery and sticky droppings (Pattison, 1987; Martinez-Amezcua *et al*., 1998; Leeson & Summers, 2005). Soybean is also deficient in biotin and methionine (Clark *et al*., 2002) therefore, further challenging skin structure. These conditions could certainly predispose birds to contact dermatitis and other ulcerative lesions (Jensen *et al*., 1970; Harms *et al*., 1977; Mayne *et al*., 2006a). Another important feed ingredient in the UK i.e. wheat, is also known to be deficient in biotin and contains considerable amount of major NSP arabinoxylans which has anti-nutritive effects and produces diarrhoea (Santos *et al*., 2004).

The higher water holding capacity of wheat bran due can reduce digesta retention time in the GIT of rats (Hori *et al.*, 2000). Traynham *et al*. (2007) reported the effects on WHC of wheat flour when replaced partially by soy flour. They indicated that for each 20g replacement of wheat flour with soy flour in 1 kg flour there was an increase of WHC by 10g/kg of the tested sample. This indicated that wheat, in combination with soybean meal, caused an increase in the digesta WHC of bird's digestive tract, probably due to complex formation between protein and carbohydrates.

Biogenic amines are biogenic substances with an amine group (e.g. histamine, cadaverine, putrecine, spermine and spermidine) formed as result of microbial decarboxylation of amino acids present in animal protein sources (Barnes *et al.*, 2001). Protein by-product meals produced especially from spoiled fish often contain biogenic amines, histamine and tyramine. These can produce diarrhoea if they exceed 10 mg/kg in the diet. Histamine is responsible for reduction in Na^+ movement in GIT and therefore, increases intestinal fluid movement. Histamine also causes irritation to intestinal lumen and ultimately results in diarrhoea. Whereas tyramine increases the production of noradrenaline which decreases the GIT motility and decrease secretory activity

Higher inclusion of molasses in poultry feed can result in electrolyte imbalance which leads towards higher moisture in faeces, due to higher K^+ and magnesium (Mg^{++}) (Ross, 1960; Leeson & Summers, 2005).

1.10.1.3.6 Deficiency of dietary components

Certain dietary components that have been identified for their role in maintaining skin integrity and foot pad quality includes trace minerals (zinc), amino acids (methionine, cystine) and vitamin (biotin, riboflavin, pantothenic acid). The deficiency of these dietary components can increase the risk for FPD and studied extensively e.g. biotin (Patrick *et al*., 1942; Harms & Simpson, 1975; Whitehead & Bannister 1981; Clark *et al*., 2002), riboflavin (McGinnis & Carver, 1947), pantothenic acid (Kratzer & Williams, 1948), methionine (Chavez & Kratzer, 1972), sulphur containing amino acids methionine and cystine (Murillo & Jensen, 1975) and zinc (Hess *et al*., 2001).

1.10.1.3.7 Interaction between water and medication

Coccidiostats are chemical agents mainly used in poultry feed to inhibit or minimize the pathogenic coccidia and to improve the immune status of the bird (Hooge *et al.*, 1999). Fracesch & Brufau (2004) reported coccidiostats, combined with electrolytes such as Na^+, K^+ and Cl^- can result in an increased moisture excretion. Ouart *et al*. (1995) have studied different levels and forms of coccidiostats and concluded that birds fed diets containing lasalocid have the highest level of water intake and excretion. The authors considered that this was a result of the increase in sodium intake.

1.10.1.3.8 Interaction between water and mycotoxins

Mycotoxins are known to produce nephrotoxicity e.g. ochratoxins, citrinin and oosporein that can cause hyperplasia of the tubular epithelium as well as nephritis (Qureshi, 1998),

diarrhoea and can induce morphological changes in the intestine. The mycotoxin citrinin can exert toxicity on the kidneys and gastrointestinal tract compromising renal function and increasing water intake and urinary excretion (Gustavson *et al.*, 1981; Leeson & Summers, 2005).

1.10.1.3.9 Feed processing

Almost all the ingredients used in poultry diets undergo some type of processing to improve nutrient release and utilisation by the bird (Lilburn, 1996). Processing helps to enhance the palatability, the bioavailability of some nutrients and can also destroy some anti-nutritional factors of the poultry diet by ensuring proper storage, increasing the surface area for uniform distribution and ensuring mixing of the nutrients (Owens & Heimann, 1994). Processing also includes the addition of chemical substances like enzymes, vitamins, minerals, antioxidants and mycotoxin binders to further enhance nutrient balance and to reduce the anti-nutritional properties of the diet.

However some processing can affect reduce nutrient utilisation by the bird which can lead in turn to wet litter. Carre *et al*. (1995) believe that birds, fed pelleted diets rather than mash one, had more moisture in the faeces. This was explained by Cowieson *et al*. (2005) that heat treatment of feed stuffs during pelleting can solubilise NSP, which can increase their anti-nutritional properties such as increasing viscosity in the GIT. According to the authors this increase in anti-nutritional properties of the diet can result in watery dropping, which can be addressed by adding exogenous fibre degrading enzymes in the diet.

Likewise fine grinding of wheat can result in watery droppings (Svihus *et al.*, 2004), although in a previous study by Svihus *et al*. (2002) they did not find any positive effects on nutrient digestibility of feeding whole wheat. Eley & Hoffman (1949) reported no correlation between feed particle size and excreta moisture contents although, according to the authors, dietary protein levels might have a significant effect on excreta moisture content as compared to particle size.

1.10.1.3.10 Effect of exogenous enzymes on water utilisation

Unlike ruminants, birds do not have a specialised microbiota capable of utilising a wide range of feed components. The use of exogenous enzymes to facilitate chemical reactions, which are otherwise either very slow or impossible, therefore, is commonplace (Kies *et al.*, 2002). As a result of a better understanding of the digestive physiology of poultry and the limitation of certain feed ingredients, the role of enzymes has increased significantly in recent years. Enzyme addition can also help to reduce feed cost, provide

flexibility in formulations, reduce environmental pollution (Choct, 2006) i.e. excessive P^+ in the faeces (Rezaei *et al.*, 2007) and improve the digestibility of fat and protein.

Some studies (Bedford, 1995; Nagaraj *et al.*, 2007a; Manning *et al.*, 2007b) have also supported the view that the addition of enzymes can help control 'wet droppings' providing the enzyme activity is matched to the substrate concentration (Choct, 2006). Maguire *et al*. (2006) reported significantly higher moisture in the manure from birds fed phytase, this could be due to additional ion release from digesta in GIT due to phytase action. So Cowieson *et al*. (2004) suggest that since phytase normally releases excessive minerals in GIT there is a need to readjust the dietary mineral levels to reduce the chances of wet litter. Whereas some studies reported no relationship of enzyme addition with higher excreta moisture content (Hughes *et al.*, 2000; Kocher *et al.*, 2002; Santos *et al.*, 2004).

1.10.1.4 **Factors associated with litter**

Good management is essential for maximum performance in poultry (Collett, 2006). This includes proper handling; vaccination and nutrition of the bird, control of in-house temperature and stocking density. The most important of all is the litter quality maintenance (Mayne, 2005) i.e. litter moisture, NH_3 and pH content must be kept under control in all circumstance.

Meat birds such as turkeys spend all their life on litter (Jodas & Hafez, 2000) therefore litter quality is of considerable importance for their welfare and more specifically in this context, skin quality hence footpad and/or leg health. Failure to achieve an acceptable litter quality can result in respiratory problems, an increase in unwanted microbial activity resulting parasitic infestation and welfare problems (Savory, 1995) that include hock burns, contact dermatitis and breast blisters. The lesions are thought to be caused by a combination of wet litter and unspecified chemical factors in the litter (Nairn & Watson, 1972; Harms *et al*., 1977; Greene *et al*., 1985; Martland, 1985; McIlroy *et al*., 1987; Schulze Kersting, 1996).

Several researchers reported a strong association between poor litter conditions and higher prevalence of FPD (Harms & Simpson, 1977; Geraedts, 1983, Martland, 1984; Martland, 1985; Ekstrand *et al*., 1997, Wang *et al*., 1998; Martrenchar *et al*., 2002, Spindler *et al*., 2005; Mayne *et al*., 2006a). The importance of litter moisture in the aetiology of is reinforced by the finding that FPD lesions may heal (Greene *et al.,* 1985), particularly, as observed by Martland (1985), when birds are moved from wet litter to dry litter. Characteristics of litter material important in control of FPD have been discussed in details in Section 1.7.1.

Litter type, depth and quality are important in the control of FPD in poultry production (Ekstrand *et al.*, 1997), also see Section 1.7.1, By understanding the importance of the litter type as described in Section 1.7.1, it becomes clear why the incidence of FPD is more prevalent with wheat straw (Ekstrand *et al.*, 1997). Pecking, scratching and turning the litter particles by the chicken can help in aeration, further reducing the particle size of the litter by breaking down the clumps. However, overuse of litter, larger size of litter particles and excessive deterioration of litter quality results in less working of the litter by the birds. Therefore, a thin layer of litter (<5 cm) results in lower levels of foot-pad dermatitis than thicker layers (Ekstrand *et al.,* 1997). A possible explanation could be that if the layer of litter is thin and less compact the chickens are more likely to peck, scratch and turn the litter particles over and thereby help to aerate the litter (Ekstrand *et al.,* 1997).

Proper ventilation of the poultry house especially in winter is another tool to control wet litter condition and FPD (Dawkins *et al.*, 2004), it can be achieved by increasing ventilation with fans and the use of side inlets, use of circulation fans within the house and ensuring an even distribution of heat in the house. But water intake can increase many fold in summer so one cannot ignore good ventilation and management in summer. As indicated by Parker *et al*. (1972) there could be an increase of 400% in water consumption if ambient temperature increases from 21.1°C to 37.8°C. Increase in water consumption due to higher ambient temperature and protein contents of the feed has also been reported by Alleman & Leclercq (1997) and Bonnet *et al*. (1997).

Drinker type, numbers and their maintenance are important in controlling wet litter e.g. nipple or cup drinkers can reduce water spillage (Bray & Lynn, 1986; Elson, 1989; Ekstrand *et al.*, 1997), using the minimum required number of drinkers (Jones *et al.*, 2005), and checking any leaks regularly. Lowering the stocking density can help reduce ammonia content by reducing the caked litter (Dozier *et al.*, 2005) as can improved air circulation at bird level (Feddes *et al.*, 2002).

Addition of clay based products in the litter can help to absorb water from the litter. Control on mechanical damage to the feet is also very important as emphasised by Wojcik *et al*. (2004) who noted that turkeys reared on slatted floors have greater damage caused to the feet as compared to those reared on a litter floor. Jensen (1985) reported higher incidences in broilers kept on wooden slats than on wire however a later study by Simpson & Nakaue (1987) did not find the same results. Sainsbury (1993) indicated that type of floor under the litter is however more important as litter on an earth floor contain almost 100g/kg more moisture than litter on a damp proof concrete floor. Even though these management practices can help, the most important aspect of controlling litter moisture content is still by controlling moisture excretion by the bird.

1.10.1.5 Enteric health and litter quality

The increase in the problem of wet litter associated with the intestinal health after the ban on in-feed antibiotics growth promoters use in poultry diets and its consequences on the increase in carcass downgrade was highlighted by Wierup (2001). Bacterial over-growth in the proximal part of the GIT gives rise to a condition known as dysbacteriosis which can cause a reduction in nutrient digestibility (e.g. reduced fat digestibility), diarrhoea and impaired intestinal health. Inefficient nutrient absorption may lead to higher microbial fermentation in the intestine (formation of biogenic amines from protein fermentation) which will irritate and damage the gut wall. It was also reported that microbial activity stimulates mucus production and viscosity in gut hence increasing the osmotic gradient from the "gut lumen to the blood" causing a reduction in water absorption and resulting in watery faeces (Van der Klis & Lensing, 2007).

According to, Diarrhoea may occur as a result of infections that cause the sick birds to drink more water (Pattison, 1987). These disorders include infectious stunting syndrome, coccidiosis and enteritis (Hemminga & Vertommen, 1985; Pattison, 1987). Protozoan "coccidian" of the species Eimeria are known to cause enteritis and diarrhoea. *Campylobacter jejuni* in the intestine has been shown to coincide with the sudden appearance of wet litter conditions (Neill *et al*., 1984). Likewise *Escherichia Coli* (E coli), may have an indirect effect upon litter quality (Pattison, 1987). Kaldhusdal & Lovland (2000) suggested that the ban on the use of in feed antibiotic growth promoters was most significant on the increase in the incidences of necrotic enteritis. Necrotic enteritis occurs frequently in houses with areas of wet litter (Collet, 2004; Hermans & Morgan, 2007), as high water activity in wet litter could possibly acytivate the dormant *Clostridium perfringens* (*C. Perfringens*) spores and increase the proliferation of *C. Perfringens*. Damp litter may also contribute to proliferation of toxic fungi and Page *et al*. (1976) have demonstrated that fungus is capable of producing dermatitis lesions of the thigh and breast.

1.10.1.6 Factors associated with environment

Climatic conditions influence litter quality, with high relative humidity both outdoors (Payne, 1967a; McIlroy *et al.,* 1987) and inside the house being associated with poor litter quality (Payne, 1967a; Weaver & Meijerhof, 1991). Therefore the effective control of humidity, temperature and air movement within the house is essential for the maintenance of litter quality. Poor management practices like ineffective ventilation systems and improper insulation can result in wet litter conditions. Therefore a combination of insulation and good ventilation are needed to keep relative humidity levels low, to encourage the evaporation of litter moisture, and to prevent the condensation on indoor surfaces which

occurs at relative humidity greater than 80% (Sainsbury, 1983). McIlroy *et al.* (1987) stated that although the ventilation capacity might be good, adequate ventilation is often constrained by the desire to conserve heat which frequently leads to a humid atmosphere with associated wet litter conditions. Weaver & Meijerhof (1991) suggested that increasing levels of internal air circulation above 24.5cm/s might have a marked effect on litter quality and reduce the incidence of breast and foot-pad lesions while inadequate ventilation increases the rate of ammonia production or other unspecified corrosive substances (Nairn & Watson, 1972; Martland, 1985). It has been said by some that ammonia concentration should not routinely exceed 20 - 25 ppm at bird broiler level (Kristensen & Wathes, 2000). However, a later study by Jones *et al*. (2005) suggested that ammonia is aversive at concentrations above approximately 10ppm. Irrespective of the absolute value in general elevated ammonia levels in poultry houses are associated with increased respiratory disease and can causes HB and irritation to the skin of footpad resulting in FPD (Harms *et al*., 1977; Harms & Simpson, 1977; Martland, 1985; Mayne *et al* 2006a).

1.11 **Summary of Literature review**

A bird gets water from three sources i.e. drinking water, water as a part of feed and metabolic water. All biochemical reactions with in bird's body require water. But an enhanced intake of water (of normal requirement) can produce wet litter condition which is correlated with FPD and HB. The key risk factors for excessive water intake and excretion in poultry are associated with feed volume, nutrients such as proteins, carbohydrates, fats and minerals, their intake and digestibility. Birds have a small GIT when compared to larger animals so any osmotic disturbance due to excessively increased concentrations of nutrient i.e. protein, carbohydrate, fats and minerals, in the GIT can result in excreta with a higher moisture content.

The most important feature of these nutrients which can increase osmolality of the digesta and absorption through birds GIT and, therefore, affect water intake and excretion are as follows: Dietary protein is probably the most important dietary factor, and specific proteinaceous factors are the source of the protein as well as the balance of its amino acids. Dietary mineral levels and their interaction with each other can have significant effect. Dietary carbohydrate structure and chemical composition e.g. type and NSP content etc, source and level of fats.

Some management practice can contribute to control of this situation and stress on the bird e.g. litter management, proper ventilation and drinker management. But most significant of all, is to control moisture excretion by the bird which can be achieved by

controlling nutrient balance and intake through nutritional modification and all this can help control FPD problem.

In view of these interacting dietary factors, dietary manipulation promises to be an important way of improving litter quality and therefore reducing FPD in turkey. The efficacy of such dietary interventions in improving litter quality is the objective of the experiments reported here.

Chapter 2

The effect of nutrient density dilution in turkey diets on water intake and excretion

2 Aim

The main objectives of this part of the project were:

To examine the effects of different dietary nutrient densities on water intake and excretion when fed to turkeys from 7 to 28 days of age and;

To examine whether dietary supplementation with exogenous phytase (by provoking a mineral imbalance) would influence water intake and excretion.

The effects of dietary nutrient densities and supplementary phytase on turkey growth performance, nutrient digestibility and apparent metabolisable energy (AME) were also examined.

2.1 Background

A change from animal to vegetable protein makes it more difficult to formulate balanced diets for poultry (Nagaraj *et al.*, 2007b). To overcome this uncertainty and to make sure that all birds receive the required nutrients, nutritionists tend to formulate diet which exceeds the actual requirement. This over supply of nutrients can change the osmotic environment within a bird's body and, especially in the GIT and, therefore, can affect the normal physiological requirement for water and can result into higher excreta moisture content. The studies done in the past on varying nutrient densities were mainly focused on performance goals. So there is a need to evaluate if changing nutrient densities without altering the ratio have any impact on water intake and excretion by turkeys.

Phytase addition studies have tended to be focused on evaluating the effect at different phosphorus levels. So there is a need to evaluate the effect of phytase supplementation on water intake and excretion when birds are fed diets with different nutrient densities.

2.2 Material and methods

2.2.1 House preparation

Prior to the reception of poults the house was vacant and thoroughly cleaned. This included proper washing and disinfection of the room. A foot dipping tank was in place at all times on the door step of the house to maintain biosecurity.

2.2.2 Experimental diets

In the pre-study period, from 0 to 7 days of age, the birds were fed a standard mash starter turkey feed (Table 1). The starter diet consisted of major feed ingredients such as wheat, soybean meal, and fish meal and had a crude protein content of 280 g/kg and an AME 12.13 MJ/kg on as it basis.

Eight experimental diets in total were used in the study. A nutritionally complete wheat-soybean basal feed (T1) was formulated according to the breeder recommendation (Aviagen Turkeys Ltd., UK). Four different levels of washed sand (0, 38.5, 74.1 and 107.1 g/kg) were added to the basal diet in replacement for feed, producing four diets in total, T1, T2, T3 and T4, respectively. Then each of the four diets were divided in two equal parts and one of the part was supplemented with exogenous *Esherichia coli*-derived phytase (Phyzyme^{TM} XP, EC 3.1.3.26 (type 6); Danisco Animal Nutrition, Wiltshire, UK) at 500 units (FTU) of phytase per kilogram diet, making another four diets T5, T6, T7 and T8 containing 0, 38.5, 74.1 and 107.1 g/kg sand plus 500 FTU each diet, respectively, giving eight dietary treatments in total. Dietary phytase was mixed with a small portion of feed using a small mixer (A200, Hobart Manufacturing Co, Ltd., London), although a bigger horizontal mixer (Helicon® Series 3, England) was used for any nutrient density dilution of the feed with washed sand and mixing of the portion of feed containing phytase. The mixing of feeds was done for 10 minutes each so as to get uniform distribution of diluting agent and enzyme in feeds. Feeds were mixed following the order of less nutrient density dilution first so that to avoid any cross contamination. All diets were offered as mash.

Table 1: Ingredient composition (g/kg) of the starter diet fed to the turkeys during the pre-study period from 0 to 7 days of age.

Ingredients	g/kg
Wheat	497.3
Fish meal - (72%-CP)	36.0
Soybean meal - (48%-CP)	400
Soy oil	25.5
Salt	2.7
DL Methionine	1.6
L Lysine	0.4
Limestone	12.9
Dicalcium phosphate	18.7
Vitamin/Mineral premix[1]	4.9
Total	1000
Calculated nutrient analysis	
Metabolisable energy (ME,MJ/kg)[2]	12.13
Crude protein (CP,g/kg)	280
Crude fibre (g/kg)	25.8
Fat (g/kg)	41.8
Ca (g/kg)	12
Available Phosphorus (g/kg)	6
Na (g/kg)	1.7
Cl (g/kg)	2.8
K (g/kg)	10.8
Lysine (g/kg)	16.2
Methionine(g/kg)	6
Metionine + Cystine (g/kg)	10.5
Threonine (g/kg)	10.5

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diet): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).

Table 2: Ingredient composition of experimental diets fed to the birds from 7-28 days of age.

Ingredient composition	Nutrient densities (% of diet)			
	100 (T1)	96.15 (T2)	92.59 (T3)	89.29 (T4)
	g/kg			
Wheat	497.3	478.2	460.5	444.0
Fish meal - (72%- CP)	36.0	34.6	33.3	32.1
Soybean meal - (48%-CP)	400	384.6	370.4	357.2
Soy oil	25.5	24.5	23.6	22.8
Salt	2.7	2.6	2.5	2.4
DL Methionine	1.6	1.5	1.5	1.4
L Lysine	0.4	0.4	0.4	0.4
Limestone	12.9	12.4	11.9	11.5
Dicalcium phosphate	18.7	18.0	17.3	16.7
Vitamin/Mineral premix[1]	4.9	4.7	4.5	4.4
Sand	0	38.5	74.1	107.1
Total	1000	1000	1000	1000
Calculated nutrient analysis				
Metabolisable energy (ME,MJ/kg)[2]	12.13	11.66	11.23	10.83
Crude protein (CP,g/kg)	280	269.2	259.3	250
Crude fibre (g/kg)	25.8	24.8	23.9	23.0
Fat (g/kg)	41.8	40.2	38.7	37.3
Ca (g/kg)	12	11.5	11.1	10.7
Available Phosphorus (g/kg)	6	5.8	5.6	5.4
Na (g/kg)	1.7	1.6	1.6	1.5
Cl (g/kg)	2.8	2.7	2.6	2.5
K (g/kg)	10.8	10.4	10.0	9.6
Lysine(g/kg)	16.2	15.6	15.0	14.5
Methionine (g/kg)	6	5.8	5.6	5.4
Metionine + Cystine (g/kg)	10.5	10.1	9.7	9.4
Threonine (g/kg)	10.5	10.1	9.7	9.4

[1]The vitamin and mineral premix (Target Feed Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).

Table 3: Analysed composition of experimental diets and sand.

Determined values	Nutrient densities (% of diet)			
	100 (T1)	96.15 (T2)	92.59 (T3)	89.29 (T4)
Dry Matter (DM,g/kg)	853	863	870	872
Crude protein (CP,g/kg)	264	254	246	240
Gross energy (MJ/kg)	16.55	15.88	15.35	15.39
Ash (g/kg)	70.3	98.5	128.2	160.7
Sand				
Dry matter (DM,g/kg)	998			
Ash (g/kg)	999.9			

2.2.3 Analysis of feed and excreta samples

Dry matter (DM) in feed and excreta was determined by drying at 100°C for 24 h in a force draft oven (AOAC 925.10, 1990). The DM in samples was obtained by the following equations:

DM (kg/kg) = 1 – sample moisture (SM, kg)

SM (kg/kg) = (SW before drying – SW after drying)/SW before drying

SW = sample weight

Equation 5: Equations for determination of dry matter and moisture in feed and excreta.

Ash in feed and excreta was measured in a muffle furnace at 500°C for 18 h. The ash (kg/kg) in the samples was determined as follow:

Ash (kg/kg) = (Weight of ash in crucible (g) / Initial weight of sample (g DM)) * 1000

Organic matter (OM) in feed and excreta was determined as difference between their DM and ash contents.

Equation 6: Equations for determination of ash and organic matter in feed and excreta.

The nitrogen content of feed and excreta was determined using a Leco nitrogen analyser (Leco FP-428, Leco Corporation, USA) according to the AOAC method 968.06 (2000). Approximately 0.15-0.2 g sample is weighed out accurately (to the nearest 0.1 mg) into a foil cup and then placed in an auto sampler. This sample is then dropped into a furnace at 850°C in the presence of pure oxygen for combustion. The sample combustion gases are then filtered and cleaned up through a steel wool particle filter and with various chemicals to provide a Nitrogen and Helium mix that is then passed through aliquot doser (detector) and carried out through a heater by a carrier gas where the Nox gasses are reduced to N_2. The instrument then provides a result for nitrogen by detecting it through a thermal conductivity cell. Crude protein (CP) values were calculated from that of nitrogen on the basis of assumption that all food protein contain 160 g N/kg, so CP values were obtained simply by multiplying the nitrogen concentration (CP (g/kg) = g N/kg x 1000/160) by 6.25.

2.2.4 Gross energy (GE) (MJ/kg)

The quantity of heat resulting from the complete oxidation of unit weight of a food or excreta is known as the gross energy (GE) or heat of combustion of that food or excreta. Gross energy of the diets and excreta was determined by an isoperibol bomb calorimeter (Model-6200 Parr Isoperibol bomb calorimeter, Parr Instruments Company, USA). The bombs were standardized by determining the heat capacity of each bomb using pellet benzoic acid standard each day at the beginning of gross energy determination processes. The constant gross energy of benzoic acid was 26.454 MJ kg^{-1}. The GE results from feed and excreta were used for calculation of dietary metabolisable energy.

2.2.5 Comparison of turkey growth performance and Apparent Metabolisable Energy determination

Two hundred and ten days old male turkeys (BUT 8) were weighed to get the initial weight and placed in experimental house located at the ASRC, SAC, Auchincruive, Ayr. For the first 7 days, birds were placed in the floor pen containing 10 cm thick bedding material of wood shaving. Birds were offered a standard turkey starter mash diet (Table 1) for the first 7 days and had *ad libitum* access to feed and water.

At seven days of age two-hundred turkeys were transferred to 40 wire-mesh metabolism cages (0.35 x 0.35 m/cage floor area), stratified on body weight, 5 birds in a cage. The cages were arranged in five tier levels with each tier serving as a block, within a controlled environment room. All the cages were equipped with metal feeders and drinkers (troughs). Excreta samples were collected in trays under each cage. The experimental diets were then introduced to the turkeys, as each dietary treatment was fed to 5 replicate cages. Each dietary treatment was replicated 5 times. Feed and water were available *ad libitum* throughout the experiment. The average air temperature of the house was recorded every day and was maintained at 30°C for 7 days and gradually reduced to maintain at 22°C till the age of 28 days. A lighting schedule of 23 hour light and 1 hour dark period was used throughout the trial. Feed intakes and growth rates were measured each week for the whole feeding period, from 7 to 28 days of age. The experiment ended when the birds were 28 days of age.

The apparent metabolisable energy (AME) of the experimental diets was determined by total collection as excreta were collected quantitatively for the last 2 days of the feeding period and immediately oven dried at 80°C. The feed intake for the same period was also measured. The GE of each dried excreta sample and the experimental diets were

determined. Dietary AME was estimated using the GE values of feed and excreta by following the equation.

$$\text{AME (MJ/kg)} = \frac{\text{GE in feed x feed intake (kg) - GE in excreta x excreta output (kg)}}{\text{Feed intake (kg)}}$$

Equation 7: Equation for the calculation of apparent metabolizable energy.

The AME of feed will vary depending on whether the amino acids it supplies are retained by the birds for protein synthesis or are deaminated and their nitrogen excreted. For this reason, AME values are sometimes corrected to zero nitrogen balance, by deducting 34.39 J for each 1 gram of nitrogen retained (Hill & Anderson, 1958).

$$\text{AMEn (MJ/kg)} = \frac{\text{(GE in feed x feed intake(kg) – GE in excreta x excreta output (kg)) - (NR x F)}}{\text{Feed intake (kg)}}$$

Where,

NR = Nitrogen retention (Nitrogen fed – Nitrogen excreted (g))

F = 34.39 MJ kg^{-1}

Equation 8: Equations for the calculation of apparent metabolizable energy corrected for nitrogen.

2.2.6 Feed intake determination

To determine the feed intake, the feed offered at the beginning of each week was recorded and the weigh back was taken at the end of each week. For the last two days of the trial, feed was recorded separately to get the feed intake for two days for digestibility determination. The values of daily feed intake were recorded and converted to a DM basis (feed intake g DM /bird/day).

2.2.6.1 **Organic matter intake and retention determination**

The feed intake and excreta organic matter content were determined by correcting for ash intake and excretion and by using the following equation. The organic matter retention (OMR) was determined by calculating the difference between organic matter intake (as a part of the feed) organic matter excreted (as a part of the excreta). The organic matter of the sand was also determined.

OMI (g) = DM intake (g) – Ash in the diet (g)

OMEx (g) = DM excreted (g) – Ash in the excreta (g)

OMS (g) = Sand dried (g) – Ash in the sand (g)

Where,
OMI = Organic matter intake
OMEx = Organic matter excretion
OMS = Organic matter sand

Equation 9: Equations for accounting ash part from feed intake and excreta.

2.2.7 Body weight determination

Birds were weighed (cage weight) as a group of five birds before placing them in cages to get the initial weight and then on weekly basis birds in each cage were weighed as a group to get the measurements for weekly body weight gain. This was then converted to body weight gain in g/day/bird.

2.2.8 Feed conversion efficiency, organic matter efficiency and protein efficiency ratios calculations

The feed conversion efficiency (FCE) was calculated by dividing weight gain by feed intake. The same applied for the organic matter efficiency (OME), and for the protein efficiency ratio (PER)-by calculating total protein intake as feed intake (g) x CP concentration in the diet ((g/kg)/1000).

$$FCE = \frac{\text{Body weight gain (g/b/d)}}{\text{Feed intake (g/b/d)}}$$

$$OME = \frac{\text{Body weight gain (g/b/d)}}{\text{OM intake (g/b/d)}}$$

$$PER = \frac{\text{Body weight gain (g/b/d)}}{\text{Protein intake (g/b/d)}}$$

Equation 10: Equations for calculation of feed conversion efficiency, organic matter efficiency and protein efficiency ratio.

2.2.9 Nutrient digestibility coefficients calculations

Calculations of the coefficient of apparent dry matter (with and without sand correction) and nitrogen digestibility were done by using the equations below. However, as presented in Table 3, the ash content of sand is almost 100% of it weight and since according to the WHO (1998) the ash from sand is acid insoluble. The content of acid-insoluble ash is the amount of silica present, in sand and siliceous earth this is regarded as "non-physiological" ash due to its unavailability to the animal. This means that actually the sand portion of the diets was not available to the bird and thus excreted as such. Since sand is totally indigestible (Van der Meulen *et al.*, 2008) a correction has been made for sand addition in the diet and excretion in excreta. Then these values were used for sand corrected dry matter digestibility determinations.

$$ADMD^{*} = \frac{DMin - DMout}{DMin}$$

$$ADMD^{**} = \frac{(DMin - S) - (DMout - S)}{DMin - S}$$

Where,
ADMD* = Coefficient of apparent dry matter digestibility (no sand correction)
ADMD** = Coefficient of apparent dry matter digestibility (sand correction
DMin = Dry matter content of feed consumed (g/kg/day)
DMout = Dry matter content of excreta output (g/kg/day)
DMin - S = Dry matter content of feed consumed (g/kg/day) - Sand intake (g/b/d)
DMout - S = Dry matter content of excreta output (g/kg/day) - Sand intake (g/b/d)

Equation 11: Equation for calculation of the coefficient of apparent dry matter digestibility (ADMD).

The coefficient of organic matter and nitrogen digestibility were calculated from the same equation as for ADMD, substituting OM and N for DM respectively.

2.2.10 Water intake determination

Clean fresh water was offered every day in a trough which was washed every day and placed at the front of each cage. Daily water intake was measured as a difference between the water offered and any left over by weighing the trough at both occasions. The level of the water in the trough was maintained all the time so that it would provide *ad libitum* access for the bird. If there was any need to top up the water the amount added

was recorded. The weighing balance was tared each time before use. To get the measurements of evaporative losses five water troughs with identical surface area and volume of water were placed each day at bird height and at different points within the experimental room which were out of the reach of birds. Evaporation losses were determined by the difference of initial and final weight of water then the average value was determined. The water measurements then were recorded as g/bird/day after correcting the evaporative losses. Whereas water:feed was recorded by daily water intake with daily feed intake both (g/b/d).

$$\underset{(g/b/d)}{WI} = \frac{(\text{Initial weight of W (g) -Final weight of W (g)}) + (\text{Average evaporation/d (g)})}{\text{Number of bird in the pen}}$$

Where,

WI = Water intake

W = Water

Equation 12: Equations for accounting evaporation from water intake per day.

2.2.11 Excreta moisture determination

To be able to determine the dry matter of excreta, excreta samples were collected every day from 8-28 days and each collection was placed for dry matter determination in oven for 48 hours at 80°C (Equation 5).

2.2.12 Moisture output ratio body weight gain and as a percent of water intake

To calculate the moisture output to body weight gain ratio (MO:WG), moisture output (g/b/d) determined during total collection was divided by average daily body weight gain. Whereas, MO (g/b/d) as % of WI (g/b/d) was calculated by following equations:

$$MO = \frac{\text{(Total excreta output (g/pen/d)) – (EDM (g/pen/d))}}{\text{Number. of birds/pen}}$$

$$MO:WG = \frac{MO}{WG}$$

$$\text{MO \% of WI} = \frac{MO\ (g)}{WI\ (g)} \times 100$$

Where,

MO = Moisture output (g/b/d)

WG = Body weight gain (g/b/d)

EDM= Excreta dry matter output (g/pen/d)

Equation 13: Equation for calculation of moisture output: body weight gain.

2.2.13 Statistical procedure

Statistical analyses were performed using the Genstat 11 statistical software package (IACR Rothamstead, Hertfordshire, England). A randomised complete block analysis of variance was performed and a 2 x 4 factorial structure was used to compare the main treatment factors (phytase x nutrient density dilution with sand). An orthogonal partitioning of the washed sand inclusion level (nutrient density dilution) was used to quantitatively compare the linear and quadratic regression effects. Least significant difference (LSD) was used to determine which means amongst the set of treatments means differ from the rest. Differences were reported as significant at $P<0.05$ and trends were noted when the P value was near to 0.1.

2.2.14 Animal ethics

The study was approved by an Animal Experiments Committee of the Scottish Agricultural College, Ayr.

2.3 **Results**

Analysed chemical composition of the basal diets is shown in Table 2. The analysed CP content was lower than the calculated values. Due to differences in feed DM content all intake data (nutrient and feed) was recorded on DM basis.

2.3.1 Growth performance

Overall body weight was similar to the breed standards, (i.e. 1037g vs. target of 1020g) at 28 days of age, and there was no treatment-related mortality. Dietary nutrient density dilution had no effect on body weight and weight gain (P>0.05) (Table 4), whereas, birds fed diluted diets had a higher daily feed intake (P<0.05) compared to those fed non-diluted diets, (Table 4). The response of feed intake to nutrient density dilution was a linear function (P<0.001). The group fed with diets T2, T3 and T4 were consuming 6, 13 and 16% respectively more feed than the group of birds fed with diet T1. However, birds fed diets T3 and T4 did not differ in terms of their response to feed intake. There was no difference (P>0.05) in organic matter intake (OMI) between individual groups fed different nutrient levels when ash content was accounted for. However, the response of OMI to nutrient density dilution was a linear function (P<0.05) (Table 4). Birds fed diluted diets had a lower feed conversion efficiency (FCE) (P<0.001) that was described best as a linear response (P<0.001) (Table 4). The group fed diets T2, T3 and T4 had a lower FCE which was about 4, 9 and 11% respectively lower than the group of birds fed with diet T1. However, birds fed diets T3 and T4 did not differ from each other, in terms of their FCE.

Supplementary phytase had no significant (P>0.05) effect on body weight, growth performance, dry matter (DM) and OM intakes and efficiencies and no interactions of phytase with dietary density were detected (P>0.05) (Table 4).

2.3.2 Dietary nutrients utilisation and metabolizable energy

The relationship between the dietary dry matter digestibility (DMD) coefficients and the dietary density were described best as a linear response (P=0.05), where a reduction in the nutrient density led to an increase in the dry matter digestibility. Dietary DMD and organic matter retention (OMR) also tended (P=0.06) (Table 4) to increase with the dietary nutrient density dilution. Dietary organic matter efficiency (OME) was not affected (P>0.05) by the nutrient density dilution.

Supplementary phytase did not have an effect (P>0.05) on the dietary digestibility coefficients, OMR and OME, and did not interact with the dietary nutrient densities (Table 4).

Birds fed diluted diets had higher DMD when sand was accounted for (P<0.05) that was described best as a linear response (P<0.01) (Table 4). The birds fed diet T4 had relatively higher sand corrected DMD which was about 8% higher than the group of birds fed with diet T1. Whereas, group of birds fed diets T1, T2 and T3 were not different from

each other, likewise groups fed diets T3 and T4 did not differ for sand corrected DMD. Dietary phytase did not have an effect on DMD (P>0.05) when sand was accounted for.

Dietary nutrient density dilution significantly (P<0.001) reduced the apparent metabolisable energy (AME) and apparent metabolisable energy corrected for nitrogen retention (AMEn) values of the diets. There was a significant (P<0.001) quadratic response of dietary AME and AMEn values to dietary nutrient dilution. AME and AMEn values were reduced for diet T3 (13.24 and 13.14 MJ/kg DM, respectively) and were slightly higher for diet T4 (13.43 and 13.31 MJ/kg DM, respectively). Diets T2, T3 and T4 had lower AME of approximately 8, 10 and 9% when compared to diet T1 (Table 5).

Supplementing diets with phytase tended (P=0.053) to reduce the dietary AME and AMEn values by about 0.2 MJ/kg DM (Table 5). There was no interaction (P>0.05) of phytase with nutrient densities in terms of effect on AME values.

Birds fed diluted diets had higher apparent metabolizable energy intake (AME I, MJ/b/d) (P<0.05) that was described best as a linear response (P<0.05) with increasing dietary nutrient density dilution (Table 5). The birds fed diet T4 had about 9% higher AME I as compared to the group of birds fed diet T1.

Dietary phytase did not have an effect on AME I (P>0.05). There was no phytase interaction with nutrient density dilution levels observed (P>0.05) for AME I (Table 5).

Dietary crude protein digestibility (CPD) values tended (P= 0.06) to respond in a quadratic fashion to nutrient densities as CPD was reduced in diets T2 and T3 (0.590 and 0.591, respectively) and was slightly higher in diets T1 and T4 (0.609 and 0.602, respectively) (Table 5).

There was no effect (P>0.05) of phytase on CPD values. CPD values did not differ (P>0.05) between diets with different nutrient densities. There was no phytase by nutrient densities interaction (P>0.05) on protein digestibility coefficient values (Table 5).

There was an interaction (P<0.001) between supplementary phytase and dietary nutrient density for its effect on protein efficiency ratio (PER), as birds fed non diluted diet had lower PER when phytase was present whereas, at higher nutrient density dilution the results were opposite and phytase was actually working to improve PER (Table 5). The highest PER value was recorded for diet T8 with phytase supplementation; however, diet T8 with phytase supplementation was not different from diets T1, T2, T3 and T4 with no phytase supplementation, and from diets T6 with phytase supplementation. This shows

that PER was actually improving with dietary nutrient dilution and phytase was helping at higher nutrient density dilution instead of lower or no nutrient dilution.

2.3.3 Water intake and excretion measurements

Although the main effect of nutrient density for water intake (WI) fails to reach significance (P>0.05) nonetheless there was overall linear effect of nutrient density on WI (P<0.05). The greater increase in WI was associated with lowest nutrient density and other nutrient densities were same (Table 5). It is important to note that when total water intake (TWI, preformed water in feed + WI) data was analysed the trends were similar to that of reported for WI, W:F etc., therefore the data was not presented. Bird fed diluted nutrient density diets had lower water:feed (W:F) as compared to those fed the non-diluted diet, and the response of W:F to nutrient density dilution was a linear function (P<0.01) (Table 5). Diets with densities of 100 and 96.15% had significantly higher (2.70 and 2.67 respectively) W:F as compared to diets with nutrient densities 92.59 and 89.29% (2.43 and 2.52, respectively). There was a decrease in W:F as the dietary nutrient dilution increased – about 11 and 7% respectively lowered for diets T3 and T4 as compared to diet T1. There was no difference in W:F (P>0.05) noted for phytase interaction with different nutrient densities. There was a non-significant (P>0.05) difference in water to organic matter ratio (W:OM) between groups fed different nutrient levels when ash content was accounted for (Table 5).

Phytase supplementation did not influence (P>0.05) daily water intake, water to feed and water to organic matter ratios (Table 5). There were no interactions of phytase with dietary nutrient density dilution (P>0.05) for daily water intake, W:F and W:OM ratios. Supplementary dietary phytase did not have an interaction with dietary density (P>0.05) for any effect on water to OM ratio.

The excreta output (g/b/d) was not affected by the dietary nutrient density dilution (P>0.05) (Table 6). Supplementary phytase and dietary nutrient density did not influence (P>0.05) the excreta output (Table 6). There was no phytase by dietary nutrient density interaction (P>0.05) for any effect on excreta output.

The moisture output as a percent of water intake of the birds was not affected by the dietary nutrient density dilution (P>0.05) (Table 6). However, feeding phytase tended (P=0.07) to increase moisture output as a percent of water intake when compared to diets with no phytase supplementation.

Dietary density dilution tended (P=0.09) to decrease the moisture output to body weight gain ratio (MO:WG) (Table 6). Where the highest value (1.17) was recorded for birds fed diet with 100% (T1) nutrient density diet which was numerically about 13% higher than the value (1.04) recorded for birds fed diet with 92.59% (T3) nutrient density and it was numerically almost 5 and 10% higher than birds fed diets T2 and T3 containing 96.15 and 92.59% nutrient concentrations respectively. Phytase addition increased (P=0.05) MO:WG by 9.5% when compared to non supplemented diets. Since there was only a trend for nutrient density effect on MO:WG, hence there was no interaction of phytase addition with nutrient densities was noted (P>0.05).

Birds fed higher nutrient density diets had significantly (P<0.001) higher excreta moisture content as compared to birds fed lower nutrient density diets (Table 6). The effect was best described as linear function of nutrient concentration (P<0.001) as birds fed higher nutrient density diets had higher excreta moisture content as compared to birds fed lower nutrient density diets. Birds fed on diet with 100% nutrient density (T1) produced excreta with highest moisture contents (731.9 g/kg of excreta) – almost 3, 7 and 11% higher than birds fed diets containing 96.15 (T2), 92.59 (T3) and 89.28% (T4) nutrient concentration. There was a non significant (P>0.05) effect of phytase feeding on excreta moisture content. There was no phytase by nutrient density dilution interaction (P>0.05) for excreta moisture contents (Table 6).

Table 4: Effect of nutrient density dilution on body weight ((BW) g/b), feed intake ((FI) g/b/d), weight gain ((WG) g/b/d), feed conversion efficiency ((FCE) g wt/g feed DMI), dry matter digestibility (DMD), organic matter intake ((OMI) g/b/d), organic matter digestibility (OMD), organic matter retention (OMR), organic matter efficiency ((OME) g wt/g OMI) for 7-28 days of age.

Treatment factors	BW (g)	FI (g/b/d)	WG (g/b/d)	FCE	DMD*	DMD**	OMI	OMD	OMR	OME
Phytase (FTU)										
0	1040	58.9	43.0	0.753	677.9	724.1	51.0	737.0	62.4	0.843
500	1033	58.7	42.8	0.747	669.8	716.3	50.9	734.5	61.3	0.842
SEM	18.9	1.008	0.847	0.0067	8.83	9.42	0.884	4.14	1.467	0.0054
Nutrient concentrations										
100	1009	53.4^{a}	41.4	0.794^{c}	692.3	692.3^{a}	48.9	730.7	59.0	0.847
96.15	1026	56.9^{b}	42.6	0.762^{b}	677.5	709.1^{a}	50.1	728.4	60.1	0.849
92.59	1058	61.5^{c}	43.9	0.728^{a}	668.2	729.9^{ab}	52.2	740.6	64.7	0.842
89.28	1054	63.5^{c}	43.7	0.714^{a}	657.6	749.0 ^{b}	52.5	743.3	63.5	0.832
SEM	26.7	1.426	1.198	0.0094	12.49	13.32	1.250	5.85	2.074	0.0076
Phytase (FTU) x Nutrient concentrations										
0 + 100	1052	55.6	43.2	0.788	711.4	712.2	51.0	736.3	62.4	0.848
0 + 96.15	994	55.0	41.3	0.768	682.9	714.6	48.7	733.0	58.5	0.845
0 + 92.59	1052	61.3	43.5	0.728	667.2	728.8	52.3	737.9	65.0	0.831
0 + 89.28	1061	63.7	44.1	0.726	650.2	740.7	51.9	740.8	63.6	0.848
500 +100	965	51.1	39.7	0.799	673.1	673.1	46.8	725.1	55.7	0.846
500 + 96.15	1057	58.8	43.8	0.755	672.2	703.5	51.4	723.8	61.7	0.853
500 + 92.59	1064	61.7	44.3	0.729	669.2	731.1	52.1	743.4	64.5	0.852
500 + 89.28	1047	63.4	43.4	0.703	664.9	757.4	53.1	745.8	63.4	0.817
SEM	37.8	2.016	1.694	0.0133	17.66	18.83	1.768	8.28	2.933	0.0107
Probabilities of statistical differences										
Phytase (FTU)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nutrient concentrations	NS	<0.001	NS	<0.001	NS	<0.05	NS	NS	NS	NS
Linear	NS	<0.001	NS	<0.001	P=0.05	<0.01	<0.05	P=0.06	P=0.06	NS
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phytase (FTU) x Nutrient concentrations	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Dietary DMD without sand correction and **with sand correction was determined between 26 and 28 days of age; There is a statistical significant difference when P<0.05; SEM- Standard errors of means; means within a column with no common superscript differ significantly. There were 5 observations per treatment. All intake data (nutrient and feed) was recorded on DM basis.

Table 5: Effect of nutrient density dilution on apparent metabolizable energy (AME), apparent metabolizable energy intake (AMEI), nitrogen corrected apparent metabolizable energy (AMEn), crude protein digestibility coefficient (CPD), protein efficiency ratio ((PER) wt g/g CPI), water intake (WI), water:feed ((W:F) g WI/g feed DMI) and water:organic matter (W:OM) for 7-28 days of age.

Treatment factors	AME (MJ/kg)*	AME I (MJ/b/d)	AMEn (MJ/kg)*	CPD*	PER (gain/ CP intake)	WI (g/b/d)	W:F	W:OM
Phytase (FTU)								
0	13.82	0.81	13.69	0.602	2.52	149.1	2.61	3.01
500	13.62	0.80	13.51	0.594	2.47	145.0	2.55	2.93
SEM	0.068	0.0136	0.066	0.0056	0.0160	3.04	0.034	0.039
Nutrient concentrations								
100	14.61^{c}	0.78^{a}	14.48^{c}	0.609	2.40^{a}	139.9	2.70^{c}	2.94
96.15	13.59^{bc}	0.77^{a}	13.48^{b}	0.590	2.51^{b}	147.1	2.67^{c}	3.04
92.59	13.24^{a}	0.81^{ab}	13.13^{a}	0.591	2.59^{c}	145.9	2.43^{a}	2.86
89.28	13.43^{ab}	0.85^{b}	13.31^{ab}	0.602	2.48^{b}	155.4	2.52^{b}	3.05
SEM	0.096	0.0192	0.093	0.0079	0.0227	4.30	0.048	0.055
Phytase (FTU) x Nutrient concentrations								
0 + 100	14.64	0.81	14.53	0.603	2.51^{bcd}	147.1	2.71	2.95
0 + 96.15	13.85	0.76	13.73	0.592	2.55^{cde}	144.6	2.73	3.08
0 + 92.59	13.26	0.81	13.14	0.595	2.57^{dec}	144.2	2.41	2.83
0 + 89.28	13.52	0.86	13.37	0.616	2.45^{b}	160.4	2.60	3.19
500 +100	14.59	0.75	14.43	0.615	2.28^{a}	132.7	2.68	2.93
500 + 96.15	13.33	0.78	13.23	0.587	2.47^{bc}	149.5	2.61	2.99
500 + 92.59	13.22	0.82	13.13	0.587	2.62^{e}	147.5	2.45	2.90
500 + 89.28	13.34	0.85	13.24	0.587	2.52^{bcd}	150.3	2.44	2.92
SEM	0.136	0.0272	0.132	0.0112	0.0321	6.076	0.068	0.078
Probabilities of statistical differences								
Phytase (FTU)	P=0.053	NS	P=0.053	NS	<0.05	NS	NS	NS
Nutrient concentrations	<0.001	<0.05	<0.001	NS	<0.001	NS	<0.01	P=0.07
Linear	<0.001	<0.01	<0.001	NS	<0.01	<0.05	<0.01	NS
Quadratic	<0.001	NS	<0.001	P=0.06	<0.001	NS	NS	NS
Phytase (FTU) x Nutrient concentrations	NS	NS	NS	NS	<0.001	NS	NS	NS

*Dietary AME (DM basis) and CPD were determined between 26 and 28 days of age; There is a statistical significant difference when P<0.05; SEM- Standard errors of means; means within a column with no common superscript differ significantly. There were 5 observations per treatment. All intake data (nutrient and feed) was recorded on DM basis.

Table 6: Effect of nutrient density dilution on excreta output, moisture output as percentage of water intake (MO% of WI), moisture output ratio weight gain (MO:WG) and excreta moisture content for 7-28 days of age.

Treatment factors	Excreta output (g/b/d)*	MO % of WI (g/g*100)*	MO:WG	Excreta moisture content (g/kg)
Phytase (FTU)				
0	96.3	43.3	1.05	696.7
500	101.5	48.5	1.15	698.7
SEM	3.70	2.00	0.035	5.49
Nutrient concentrations				
100	95.5	49.1	1.17	731.9^{d}
96.15	98.3	46.4	1.12	715.0^{c}
92.59	101.3	45.8	1.04	686.6^{b}
89.28	100.7	42.3	1.06	657.3^{a}
SEM	5.24	2.83	0.050	7.76
Phytase (FTU) x Nutrient concentrations				
0 + 100	90.1	42.6	1.01	727.6
0 + 96.15	92.3	44.1	1.09	720.3
0 + 92.59	99.4	44.8	1.01	682.8
0 + 89.28	103.6	41.7	1.08	656.4
500 +100	100.9	55.7	1.32	736.3
500 + 96.15	104.3	48.6	1.16	709.7
500 + 92.59	103.1	46.9	1.06	690.5
500 + 89.28	97.7	42.8	1.05	658.2
SEM	7.41	4.00	0.071	10.97
Probabilities of statistical differences				
Phytase (FTU)	NS	P=0.07	P=0.05	NS
Nutrient concentrations	NS	NS	NS	<0.001
Linear	NS	NS	P=0.09	<0.001
Quadratic	NS	NS	NS	NS
Phytase (FTU) x Nutrient concentrations	NS	NS	NS	NS

*Excreta output and moisture output as a percent of water intake were determined between 26 and 28 days of age; There is a statistical significant difference when P<0.05; SEM- Standard errors of means; means within a column with no common superscript differ significantly. There were 5 observations per treatment.

2.4 **Discussion**

Almost all the previous studies and reports aiming to investigate nutritional influence on water intake and excretion in poultry and other animals were largely designed with variation in one nutrient or ingredient. Main objectives of studies reported in literature involving phytase supplementation in poultry were to evaluate its impact on P availability and performance of the birds when diets were deficient in P concentration. However, less emphasis was placed on any potential imbalance of mineral availability in the birds GIT and its effects on water intake and excretion when a nutritionally sufficient diet would be supplemented with phytase. This indicated that perhaps the changed nutrient profile/ratios as well as any possible variation of ingredient inclusion levels in the diet had been ignored. Likewise evidences on the effect of dietary phytase supplementation on water utilisation in poultry are missing. So these situations have left some unanswered questions e.g.

- Whether the changed nutrient profile had any influence on water intake and excretion instead of a particular nutrient in question?
- Whether the changed nutrient profile had any influence on those parameters which can have confounded effects on water intake and excretion i.e. nutrient utilisation, feed intake etc?
- Whether the changes in nutrient or ingredient levels have created imbalance in certain nutrients e.g. amino acids which could have resulted in poor utilisation and gave poor results?
- Whether the changes in ingredients inclusion level have greater influence on water intake and excretion instead of a particular nutrient in question?
- Whether the imbalance in mineral availability due to phytase supplementation can also be responsible for the excessive water excretion?

Nutrient density dilution by reformulation of the diet makes it impossible to achieve an absolute balance of all the nutrients, so a practical approach to investigate variable nutrient density by diet dilution which assures the nutrient balance had to be applied to determine the impact on water intake in animal studies (Leeson *et al.*, 2001). Diets can also be diluted with some indigestible material like cellulose or sand. However, Cherry *et al*. (1983) found that the use of cellulose as a diluent in layer diets led to different performance that can be explained by the effect of cellulose on feed intake, digesta viscosity, feed passage and, possibly, changes in gut microbial population (Hartini *et al.*, 2003). This present study is amongst few to our knowledge where the nutrient profile as well as the ingredient inclusion in diets was the same. It was the concentration or density

of the nutrients which was tested to find out the effect on performance parameters and most importantly on water intake and excretion.

2.4.1 Growth performance

The present study indicates that turkeys adjust their feed consumption over a wide range of dietary nutrient density levels, in agreement with the well documented scientific literature (Payne, 1967; Morris, 1968; Leeson *et al.*, 1996, Newcombe & Summers, 1985; Van der Lee *et al.*, 2001; Leeson *et al.*, 2001; Svihus & Hetland, 2001; Van Krimpen *et al.*, 2007; Wu *et al.*, 2007; Van Krimpen *et al.*, 2009). However when feed was accounted for on the basis ash content (i.e. organic matter intake (OMI)) the intake tended to remain the same across all nutrient densities. These results agree with the conclusion of Farjo *et al*. (1986) and Nielsen (2004), Pesti & Smith (1984) and Plavnik *et al*. (1997) that, provided there is no physical constraint, birds eat to fulfil mainly their energy requirements, thereby affecting the efficiency of feed utilization. Therefore the increase in feed intake (not accounted for sand) was not commensurate with the increase in body weight gain (WG) (Rowland & Hooge, 1980; Onwudike, 1986), and hence resulted in poor FCE. Although the main effect of nutrient densities fails to reach significance ($P>0.05$) nonetheless there was over all a linear effect of nutrient densities on OMI. However WG was not as great as the increase in the OMI and hence did not improve organic matter efficiency (OME), in agreement with the findings of Saleh *et al*. (2004), and Oluyemi *et al*. (1978), Rowland & Hooge (1980) and Sahraei & Shariatmadari (2007). Conversely some scientists reported an improved feed conversion efficiency (FCE) when diet was accounted for indigestible diluting agent (Onwudike, 1986; Lee & Leeson, 2001; Yussefi Kelaricolaii *et al.*, 2001; Teimouri *et al.*, 2005; Rezaei *et al.*, 2006).

According to Bennett *et al*. (2002) qualitative diet density dilution may cause a change in the digestive physiology due to increased grinding and gut motility and therefore the increased energy requirement of the gastrointestinal tract can affect the weight gain. In the present trial the digestibility of the organic matter (OM) improved numerically and therefore an improvement in organic matter retention (OMR) was observed (possibly due to grinding in the presence of sand) which could have resulted in a reduction in nutrient load in faecal excretion. These findings were supported by findings of Skinner *et al*. (1993) who reported better feed and nutrient utilisation in broiler chickens fed lower nutrient density diets. The numerical improvement in body weight gain might be due to higher nutrient extraction i.e. higher protein efficiency ratio (PER) in the presence of diluent.

The benefits in bird performance due to phytase supplementation to the diets are well documented in the literature (Selle & Ravindran, 2007; Pirgozliev *et al.*, 2008; Pirgozliev *et*

al., 2009; Karadas *et al.*, 2010). However, the diets used in this study were phosphorus sufficient, explaining the lack of phytase effect on bird growth performance. The trend to decrease dietary apparent metabolisable energy (AME) and protein efficiency ratio (PER), and increase moisture output:weight gain (MO:WG) suggest that dietary phytase had an effect on mineral balance, e.g. releasing more available P and changing the Ca:P ratio in diets.

2.4.2 Dietary nutrients utilisation and metabolizable energy

Positive effects of nutrient density dilution with sand on performance, feed and nutrient utilisation particularly energy utilisation in poultry is well documented in literature e.g. Hooge & Rowland (1978) in layers, Hogsette *et al*. (1976) in broiler breeders, Rowland & Hooge (1980) and Farjo *et al*. (1986) in broilers and Oluyemi *et al*. (1978) and Miles *et al*. (1981) in turkey poults. A trend of better organic matter digestibility was recorded in birds fed with lower nutrient density diets (possibly an effect of sand). A small amount of nutrient intake each time due to nutrient density dilution might have made bird capable of extracting more nutrients from digesta. This could possibly be a result of slower digesta passage rate and physical separation of feed particles in the gastrointestinal tract (GIT) due to sand, which could have helped digestive juices to act more effectively. As reported by Onwudike (1986) the use of sand helped to utilise high level of crude fibre in the diet of pullets, similar findings were reported by Hogsette *et al*. (1976) and Miles *et al*. (1981). Historically proven from reports by Hill & Dansky (1954) and Mraz *et al*. (1957), that chicken can utilise lower dietary nutrient concentration more efficiently. Svihus *et al*. (2001) reported significant improvement in starch digestibility in broiler due to 10% dietary nutrient concentration dilution with cellulose and concluded that higher concentration of starch in GIT was the reason of poor digestibility in non-diluted diets. In contradiction to above when lower nutrient density diets were offered to broiler breeders Enting *et al*. (2007) observed a lower nutrient digestibility. A better PER with lower nutrient density diets were in agreement with the findings of Summers *et al*. (1964) and Marks & Pesti (1984) as they observed increased net protein utilisation at lower dietary protein levels in growing chickens. Other studies indicated similarly that dietary diluents can be used to improve energy utilisation by poultry (Mraz *et al.*, 1957; Voitle & Harms, 1976; Harms & Voitle, 1977; Hooge & Rowland, 1978).

The lack of response to phytase in terms of OM digestibility could be the reason of non availability of substrate (phytate) or masking effect of sand on phytase activity. It is also possible that the units of phytase were not enough to produce any difference. Adequate supply of phosphorus in the present trial might be another reason of no response of phytase in terms of OM digestibility and increase in excretion of endogenous material

could have contributed in slightly lower OM digestibility coefficient values. The interaction of phytase with nutrient densities for improvement in PER indicates that at higher nutrient density dilution a better dry matter digestibility (DMD) (possible positive effect of sand) could have provided some aid to phytase activity. Another possibility for improved PER due to phytase presence at higher nutrient density dilution could be due to reduction in anti-nutritional effects of phytate as described by Cowieson *et al*. (2004) for endogenous amino acid losses in broiler chickens. There is also a possible interpretation of PER that this difference in PER could be a result of simple depression for non-diluted phytase supplemented diet, may be due to imbalance of minerals in the GIT e.g. Ca:P ratio.

In the present trial the tendency of positive effects of lower nutrient density on OM and DM (accounted for sand) digestibility were in line with the findings of Van der Meulen *et al*. (2008). The reason for better nutrient utilisation was mentioned in literature (Farjo *et al.*, 1986; Hetland *et al.*, 2004) and by Nam *et al*. (1998) as the inclusion of sand in the diet resulted in a better gizzard development which then resulted in to better grinding and reduction of particle size. The results of present study provide information through a trend of better organic matter digestibility (OMD), OMR and a significantly improved DMD (sand accounted for) in group of turkeys due to nutrient concentration dilution perhaps indicated that not only these birds were retaining more OM but also mineral component of the diets. Since excreta contain up to 85% water, therefore, an increased production of excreta is observed due to higher indigestible OM results in increased accompanied water excretion and an associated water intake. This means that a higher retention of nutrients in the body therefore, would have reduced osmotic pressure in the GIT and body hence, resulted in lower moisture excretion in birds fed diets with highest nutrient density dilution.

As expected, apparent metabolizable energy (AME, MJ/kg DM) was lower when diet was diluted and this was the main reason of higher feed intake (including sand) as birds offered diets with dilutant were trying to maintain their energy requirement. As mentioned previously, birds eat to maintain their nutrient requirement especially energy, so results of AME intake provide a confirmation. However, some studies such as that of Miles *et al*. (1981) have done correction of sand from feed and excreta for the calculations of AME and CP digestibility which may not be a correct attempt as gross energy and CP was determined from the actual material (including sand) therefore, any recalculations may have resulted in higher numbers, hence we did not attempt to correct sand for AME or CPD.

2.4.3 Water intake and excretion measurements

In the present trial a lower water to feed ratio was observed in birds fed lower nutrient density diets. Although the main effect of nutrient density for water intake (WI) fails to reach significance ($P>0.05$) nonetheless there was an overall linear effect of nutrient density on WI ($P<0.05$). The greater increase in WI was associated with the lowest nutrient density and other nutrient densities were same. Therefore no difference in water intake, even at higher feed intake due to nutrient density dilution, indicates that perhaps the nutrient intake is a true indicator of water intake instead of volume of feed intake. As recorded in the present study that even though birds increased their proportional feed consumption when fed with lower nutrient density diets, the intake of nutrients e.g. protein and minerals, remained the same. Possible mechanism of water intake as described by Mroz *et al*. (1995) could be that the amount of nutrient intake stimulates the gastric secretagogues (sensory receptors in gastric mucosa) which initiate afferent impulses that in turn result in the activation of hypothalamus responsible for water intake.

As birds eat to maintain their nutrient requirement (Leeson *et al.*, 1991; Zubair & Leeson, 1994; Sahraei & Shariatmadari, 2007), so relating water intake with feed intake when nutrient density dilution is in place may not be accurate as previously reported by Larbier & Leclercq (1994) and Leeson & Summers (2005) that water intake is positively correlated with feed intake. In conclusion of the present study no significant difference in water intake was due to non significant difference in organic matter intake an indicator of nutrient intake. Study by Schutte *et al*. (1992b) indicates that water intake is linked with nutrient intake and reported higher water intake in chickens when fed diets containing L-arabinose than those fed diet without any sugar addition. Supported by findings of Pfeiffer *et al*. (1995) and Shaw *et al*. (2006) that it was the protein intake rather than feed intake which affects water intake in pigs, and by Alleman *et al*. (1997) in broilers. Likewise different studies have reported the effect of dietary mineral levels on water intake in poultry (Hooge *et al.*, 1999; Smith *et al.*, 2000a; Smith *et al.*, 2000b; Borges *et al.*, 2003a; Mushtaq *et al.*, 2005) and in pigs (Maenz *et al.*, 1994).

A lower water:feed (W:F) in birds fed lower nutrient density diets was due to the fact that these groups had higher feed intake (sand included) and similar OM intake so nutrient intake was same which was mainly responsible for water intake. Secondly, feeding birds with diluted diets can change their behaviour as reported by Van Krimpen *et al*. (2009) and this could possibly resulted in an increase in the time turkeys have spent on feeding trough rather than on water trough which therefore, resulted in lower W:F. This can help reduce excreta moisture content in the end without affecting the performance of the bird.

Significantly lower excreta moisture contents were noted in turkeys fed diets with lower nutrient densities. Findings of present trial were in line with reports of Farjo *et al*. (1986) as they observed a decrease in faecal moisture contents when birds were offered a lower nutrient density diets as compared to the groups fed non-diluted diets. Seller *et al*. (1980) and Bilgili *et al*. (2006) also reported a lower faecal moisture content with the addition of diluents in broiler and layer diets.

Son & Karasawa (2001) highlighted the importance of the lower gut in chickens for water absorption and any impairment in this important feature can result in higher water consumption and water to feed ratio. Undigested materials and excessive nutrients in large intestine can increase osmolarity of digesta (Etheridge *et al.*, 1984). This higher osmolarity is possibly the reason of lower water absorption which resulted in higher excreta moisture contents as noted in the present trial when higher nutrient density diets were fed to turkeys findings are in line with Manez *et al*. (1994) and Hooge *et al*. (1999).

Unexpectedly we did not find any difference in water to feed ratio due to the presence of phytase. The dose may have been ineffective to release higher amount of nutrients in the GIT of the birds or simply sand had a masking effect on phytase activity. There is possibility that when diets are nutritionally sufficient, supplementary phytase may not have any effect on growth and water intake, although it might increase minerals in the GIT. Higher minerals do not always increase water intake as reported by Shaw *et al*. (2006) for pigs. However, in the present study there was an indication of increase in moisture output as a percent of water intake due to the effect of phytase (P=0.07). This may be an indication of minerals being made available (not measured in the present study) in GIT due to phytase addition as indicated by Cowieson *et al*. (2004), which can result in higher osmotic pressure and therefore higher MO. This resulted in about 11% higher moisture output as a percent of water intake was recorded. The findings of present study were in line with the findings of Hooge *et al*. (1999) who recorded higher water output as percent of water intake when broilers were fed diets with higher concentration of NaCl as compared to the birds fed diet with normal NaCl concentration. Similarly moisture output ratio to body weight gain was significantly affected due to presence of phytase in the diets; there was 11% more moisture output recorded for same body weight gain due to phytase. When diets were nutritionally adequate there was no significant difference in excreta moisture content noted due to presence of phytase.

2.5 **Conclusion**

Excreta moisture content was reduced significantly as nutrient density decreased. Nutrient density had no effect on organic matter efficiency, water intake and daily weight gain,

whereas FCE and FI were higher in higher nutrient density dilution fed birds. These results clearly indicate that nutrient concentration dilution reduces excreta moisture content and has positive effects on OM and DM digestibility.

Dietary phytase tended to increase water output compared with diets without phytase, for same body weight in turkeys.

There is a need of a study to determine whether the nutrient intake or nutrient utilisation and/or a combination of both are responsible for litter quality issues in turkeys. To understand interaction of wet litter and nutrition, studies designed with changes in nutrient concentrations and ratios mainly energy and protein, on floor conditions and to evaluate the effect of litter quality parameters on welfare indicators i.e. footpad dermatitis (FPD) and hock burns (HB) will help.

Chapter 3

Effect of varying the concentrations of dietary energy and protein while maintaining a constant ratio between the two on litter moisture content and FPD in growing turkeys

3 Aim

The specific objectives of this part of the project were to assess the effect of varying dietary protein (with an ideal amino acid ratio) and metabolisable energy concentrations while keeping a constant ratio between them on:

- water intake and excretion
- litter quality
- FPD
- growth performance, dietary nutrient intake and utilisation

3.1 Background

Litter quality is an important component of many production systems but especially for broilers and meat producing turkeys as these birds stay in contact with the litter throughout their life (Ekstrand *et al.*, 1997). High litter moisture and ammonia (NH_3), content and quality are correlated with dirty footpads, footpad dermatitis (FPD) and hock burn (HB) lesions in poultry (Ekstrand *et al.*, 1997; Dawkins *et al.*, 2004; Haslam *et al.*, 2006; Mayne *et al.*, 2007). Therefore, the three most important aspects of litter quality are the moisture content, stickiness and nitrogen or NH_3 content in the litter (Lister, 2009). A good quality litter should satisfy the bird's welfare requirements by absorbing moisture, providing a warm and dry surface to rest on, providing a substrate that allows microbial activity to degrade excreta and should encourage dust bathing and litter directed activity. As shown in the first study the modification of nutrient supply affects excreta moisture content. The second study attempted to limit the confounding factors to only two i.e. apparent metabolisable energy and crude protein (AME and CP). The effect of dietary energy on feed intake is emphasised in literature which is correlated with water intake. Some reports (Collin *et al.*, 2003) suggest that achieving a higher AME to CP ratio by using a lower CP concentration might encourage birds to increase feed intake to meet their amino acid requirements, which may also increase water intake (WI) and have an impact on the litter quality. However, it is not clear whether the absolute protein concentration itself or the ratio between the dietary protein and energy was the reason for the deterioration of the litter quality or to the changes in the CP to AME ratio. Therefore,

the aim of this experiment to compare the effect on WI and litter quality (e.g. moisture content, pH and NH_3 content) of different nutrient density diets formulated to give a constant CP to AME ratio in all diets and to establish how these dietary modifications can affect litter characteristics and the correlation of these characteristics with the FPD and HB in turkeys.

3.2 **Materials and methods**

3.2.1 House preparation

See Section 2.2.1.

3.2.2 Feed preparation

In the pre-study period, from 0 to 4 weeks of age, the birds were fed a standard crumb starter turkey feed (Table 7). The starter diet consisted of major feed ingredients such as wheat, soybean meal, and fish meal containing crude protein 263 g/kg and ME 12.15 MJ/kg.

Five experimental diets in total were used for each growth phase (4 weeks each and starting at 4 weeks of age until 20 weeks) in the study. The wheat-soybean based diets in pelleted form was prepared according to the formulation for BUT 8 (Aviagen Turkeys Ltd., UK) given below (Table 9 to Table 12). Diet T3 served as control with 100% of crude protein and energy according to BUT 8 requirement for each growth phase, while diets T1, T2, T4 and T5 contained 77, 85, 110 and 120% concentration of crude protein and energy, respectively. All the diets were formulated according to the respective growth phase nutrient recommendation of BUT 8 other than protein and energy content. Digestible amino acid profile was similar during a growth phase of 4 weeks for all the diets according to BUT 8 recommendations with some missing data values for amino acids being obtained from Firman & Boling (1998) and upgraded according to commercial values (Table 8). Amino acids like lysine, methionine and threonine were included where deficient to meet the requirement. Each experimental diet for the respective growth phase was fed randomly to selected seven replicates for the period from 4 to 20 weeks. All feed was pelleted. The diets used for experiment were analysed for their dry matter (DM), crude protein (CP) minerals, crude fat (EE), crude fibre (C.fibre), ash, ME and amino acid content.

The methodology for DM, Ash, nitrogen and gross energy determinations were described in Sections 2.2.3 and 2.2.4. The fat content was determined with AOAC 920.39 method

using a Soxtec 1043 extraction unit (Foss Ltd, Wigan, UK). The dietary neutral detergent fibre (NDF) fraction was determined according to procedure described by Holst (1973).

The methodology for feed conversion efficiency (FCE) and for protein efficiency ratio (PER) is described in Section 2.2.8 however, the units for weight gain and CP intake was kg instead of g. Whereas energy efficiency ratio (EER) was calculated as weight gain (kg/d) / AME intake (MJ/d). The methodology for determination of nutrient digestibility coefficients calculations were used are described in Section 2.2.9 but for amino acid digestibility coefficients the equations were modified for each amino acid described in Section 2.2.9., and determination of parameters such as dry matter intake, excretion and retention explained in Section 2.2.6.1.

Table 7: Ingredient composition (g/kg) of the starter diet fed to the turkeys during the pre-study period from 0 to 4 weeks of age.

Ingredients	g/kg
Fish meal - (72%-CP)	30
Soybean meal - (48%-CP)	275
Wheat	575
Soy oil	17.4
Corn gluten - (60%-CP)	20
Casein	30
Lysine HCl	1.9
DL Methionine	2.8
L-Threonine	3.9
Salt	2.2
Limestone	7
Dicalcium phosphate	21.5
Vit./min. premix[1]	2.8
Coccidiostat	0.5
Pellet binder	10
Calculated nutrient analysis	
Metabolisable energy (ME), MJ/kg[2]	12.15
Crude protein (CP) (g/kg)	263.1
Crude fibre (g/kg)	29
Ca (g/kg)	10
Available Phosphorus (g/kg)	5
Na (g/kg)	1.5
Cl (g/kg)	2.3
K (g/kg)	8.2
Indispensable amino acids	
Arginine (g/kg)[3]	12.2
Cystine (g/kg)[3]	4.2
Isoleucine (g/kg)[3]	9.6
Lysine (g/kg)[3]	13.1
Methionine (g/kg)[3]	5.1
Phenylalanine (g/kg)[3]	10.5
Threonine (g/kg)[3]	8.1
Tryptophan (g/kg)[3]	3.1
Valine (g/kg)[3]	10.4
Dispensable	
Tyrosine (g/kg)[3]	9.4

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diet): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 8: Ideal protein ratios for different growth phases.

Amino acids[3]	Ideal protein ratios expressed as % relative to lysine for different growth phases			
	week 4-8	week 8-12	week 12-16	week 16-20
Arginine[1]	97.5	91.1	90.4	90.3
Cystine[1]	31.6	34.8	34.9	38.7
Isoleucine[2]	71.5	71.1	74.3	78.5
Lysine[1]	100.0	100.0	100.0	100.0
Methionine[1]	38.6	40.7	44.4	45.2
Phenylalanine[2]	78.5	77.8	76.6	74.9
Threonine[1]	61.4	60.0	60.1	60.2
Valine[2]	77.8	77.8	72.2	70.1
Tryptophan[1]	24.1	23.0	22.8	22.6
Tyrosine[2]	70.3	69.6	68.7	66.3

[1]From Aviagen Turkeys Ltd., UK.
[2]From Firman & Boling (1998).
[3]The ratios between amino acids were calculated on the basis of digestible concentration of each amino acid.

Table 9: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 4-8 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	193.0	229.7	297.3	341.8	386.2
Wheat, White	449.6	426.8	384.8	357.2	329.6
Wheat Middlings	150.00	121.50	69.00	34.50	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Corn gluten meal - (60%-CP)	0.00	1.90	5.40	7.70	10.00
Casein	0.00	9.50	27.00	38.50	50.00
Soybean OiL	0.00	23.85	67.77	96.64	125.50
L-Lysine HCl	3.40	2.75	1.56	0.78	0.00
DL-Methionine	2.50	2.75	3.20	3.50	3.80
L-Threonine	3.30	3.64	4.27	4.69	5.10
Common Salt	2.30	2.28	2.25	2.22	2.20
Limestone	12.20	10.72	7.99	6.19	4.40
Dicalcium phosphate	20.00	19.91	19.73	19.62	19.50
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	9.72	10.61	12.26	13.35	14.43
Crude protein (g/kg)	201.4	222.4	261.1	286.6	312.0
Crude fibre (g/kg)	54.30	48.92	39.02	32.51	26.00
Ca (g/kg)	10.00	9.98	9.95	9.92	9.90
Available Phosphorus (g/kg)	5.00	5.00	5.00	5.00	5.00
Na (g/kg)	1.50	1.50	1.50	1.50	1.50
Cl (g/kg)	2.50	2.41	2.23	2.12	2.00
K (g/kg)	8.90	9.01	9.22	9.36	9.50
Mn (mg/kg)	105.7	100.4	90.5	84.0	77.5
Zn (mg/kg)	105.0	99.9	90.5	84.3	78.1
Indispensable amino acids					
Arginine (g/kg)[3]	10.10	11.13	13.02	14.26	15.50
Cystine (g/kg)[3]	3.20	3.54	4.17	4.59	5.00
Isoleucine (g/kg)[3]	6.70	7.65	9.40	10.55	11.70
Lysine (g/kg)[3]	10.20	11.28	13.28	14.59	15.90
Methionine (g/kg)[3]	3.90	4.32	5.09	5.59	6.10
Phenylalanine (g/kg)[3]	7.10	8.13	10.02	11.26	12.50
Threonine (g/kg)[3]	6.20	6.87	8.09	8.90	9.70
Tryptophan (g/kg)[3]	2.50	2.75	3.20	3.50	3.80
Valine (g/kg)[3]	7.30	8.38	10.38	11.69	13.00
Dispensable					
Tyrosine (g/kg)[3]	6.20	7.17	8.95	10.13	11.30

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 10: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 8-12 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	5.70	16.20	23.10	30.00
Soybean Meal - (48%-CP)	80.0	124.7	206.9	261.0	315.0
Wheat, White	510.6	491.8	457.1	434.4	411.6
Wheat Middlings	200.00	162.00	92.00	46.00	0.00
Wheat Bran	150.0	121.5	69.0	34.5	0.00
Corn gluten meal - (60%-CP)	0.00	3.80	10.80	15.40	20.00
Casein	10.00	13.80	20.80	25.40	30.00
Soybean OiL	0.00	27.65	78.57	112.04	145.50
L-Lysine HCl	3.50	3.18	2.58	2.19	1.80
DL-Methionine	2.40	2.69	3.21	3.56	3.90
L-Threonine	1.80	2.31	3.26	3.88	4.50
Common Salt	1.30	1.34	1.41	1.45	1.50
Limestone	10.70	9.71	7.89	6.70	5.50
Dicalcium phosphate	16.00	16.19	16.54	16.77	17.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.04	11.00	12.77	13.94	15.10
Crude protein (g/kg)	169.0	187.2	220.7	242.8	264.8
Crude fibre (g/kg)	50.30	45.63	37.02	31.36	25.70
Ca (g/kg)	8.50	8.50	8.50	8.50	8.50
Available Phosphorus (g/kg)	4.20	4.20	4.20	4.20	4.20
Na (g/kg)	1.20	1.18	1.15	1.12	1.10
Cl (g/kg)	1.90	1.88	1.85	1.82	1.80
K (g/kg)	7.60	7.73	7.98	8.14	8.30
Mn (mg/kg)	106.3	100.4	89.4	82.2	75.0
Zn (mg/kg)	106.9	100.5	88.6	80.8	73.1
Indispensable amino acids					
Arginine (g/kg)[3]	8.10	8.97	10.58	11.64	12.70
Cystine (g/kg)[3]	3.00	3.32	3.92	4.31	4.70
Isoleucine (g/kg)[3]	5.80	6.52	7.85	8.73	9.60
Lysine (g/kg)[3]	8.70	9.63	11.35	12.47	13.60
Methionine (g/kg)[3]	3.60	3.94	4.57	4.99	5.40
Phenylalanine (g/kg)[3]	6.10	6.96	8.53	9.57	10.60
Threonine (g/kg)[3]	5.30	5.87	6.92	7.61	8.30
Tryptophan (g/kg)[3]	2.10	2.31	2.69	2.95	3.20
Valine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Dispensable					
Tyrosine (g/kg)[3]	5.20	6.00	7.47	8.43	9.40

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 11: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 12-16 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	9.50	27.00	38.50	50.00
Soybean Meal - (48%-CP)	41.70	70.83	124.48	159.74	195.00
Wheat, White	614.7	598.5	568.8	549.2	529.6
Wheat Middlings	144.2	116.8	66.3	33.2	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	7.60	21.60	30.80	40.00
Soybean OiL	0.00	27.1	77.1	109.9	142.7
L-Lysine HCl	4.90	4.37	3.39	2.74	2.10
DL-Methionine	2.80	3.10	3.66	4.03	4.40
L-Threonine	2.10	2.42	3.02	3.41	3.80
Common Salt	1.40	1.38	1.35	1.32	1.30
Limestone	9.00	7.56	4.90	3.15	1.40
Dicalcium phosphate	15.50	15.60	15.77	15.89	16.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.44	11.38	13.12	14.27	15.41
Crude protein (g/kg)	146.5	162.2	191.1	210.0	229.0
Crude fibre (g/kg)	47.70	43.24	35.01	29.61	24.20
Ca (g/kg)	7.50	7.50	7.50	7.50	7.50
Available Phosphorus (g/kg)	3.80	3.80	3.80	3.80	3.80
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.30	2.22	2.08	1.99	1.90
K (g/kg)	6.70	6.66	6.59	6.55	6.50
Mn (mg/kg)	100.4	95.2	85.6	79.3	73.0
Zn (mg/kg)	98.93	93.84	84.45	78.29	72.12
Indispensable amino acids					
Arginine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Cystine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Isoleucine (g/kg)[3]	4.70	5.40	6.70	7.55	8.40
Lysine (g/kg)[3]	8.10	8.96	10.53	11.57	12.60
Methionine (g/kg)[3]	3.60	3.98	4.68	5.14	5.60
Phenylalanine (g/kg)[3]	5.00	5.74	7.11	8.00	8.90
Threonine (g/kg)[3]	5.20	6.02	7.52	8.51	9.50
Tryptophan (g/kg)[3]	1.70	1.87	2.19	2.39	2.60
Valine (g/kg)[3]	5.20	5.77	6.82	7.51	8.20
Dispensable					
Tyrosine (g/kg)[3]	4.30	5.00	6.30	7.15	8.00

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 12: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 16-20 weeks of age.

Ingredients	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%-CP)	0.00	11.31	32.13	45.82	59.50
Soybean Meal - (48%-CP)	0.00	25.3	71.9	102.6	133.2
Wheat, White	639.6	630.0	612.2	600.5	588.8
Wheat Middlings	169.60	137.38	78.02	39.01	0.00
Wheat Bran	150.00	121.50	69.00	34.50	0.00
Casein	0.00	5.70	16.20	23.10	30.00
Soybean OiL	0.00	29.83	84.78	120.89	157.00
L-Lysine HCl	3.20	2.59	1.47	0.74	0.00
DL-Methionine	1.60	1.83	2.25	2.52	2.80
L-Threonine	0.20	0.39	0.74	0.97	1.20
Common Salt	1.40	1.34	1.24	1.17	1.10
Limestone	8.20	6.64	3.77	1.89	0.00
Dicalcium phosphate	12.50	12.54	12.61	12.65	12.70
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME, MJ/kg[2]	10.48	11.52	13.43	14.69	15.95
Crude protein (g/kg)	129.5	142.5	166.5	182.3	198.0
Crude fibre (g/kg)	48.70	43.93	35.15	29.37	23.60
Ca (g/kg)	6.50	6.52	6.55	6.58	6.60
Available Phosphorus (g/kg)	3.20	3.16	3.09	3.05	3.00
Na(g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	1.90	1.81	1.63	1.52	1.40
K (g/kg)	6.20	6.09	5.88	5.74	5.60
Mn (mg/kg)	101.3	95.6	84.9	78.0	71.0
Zn (mg/kg)	100.8	95.2	84.8	78.0	71.1
Indispensable amino acids					
Arginine (g/kg)[3]	5.70	6.33	7.48	8.24	9.00
Cystine (g/kg)[3]	2.30	2.55	3.00	3.30	3.60
Isoleucine (g/kg)[3]	4.20	4.75	5.77	6.43	7.10
Lysine (g/kg)[3]	6.00	6.65	7.84	8.62	9.40
Methionine (g/kg)[3]	2.80	3.09	3.61	3.96	4.30
Phenylalanine (g/kg)[3]	4.50	5.11	6.23	6.96	7.70
Threonine (g/kg)[3]	3.50	3.90	4.63	5.12	5.60
Tryptophan (g/kg)[3]	1.50	1.63	1.88	2.04	2.20
Valine (g/kg)[3]	4.70	5.37	6.59	7.40	8.20
Dispensable					
Tyrosine (g/kg)[3]	3.80	4.39	5.47	6.19	6.90

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 13: Analysed composition of experimental diets for 4-8 weeks growth phase.

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	868.8	868.9	869.2	869.3	869.5
Crude protein (g/kg)	193.2	215.7	257.2	284.4	312.1
Gross energy (MJ/kg)	16.27	16.77	17.70	18.31	18.94
Ash (g/kg)	64.74	64.92	65.26	65.48	65.77
Crude fat (g/kg)	30.24	46.95	77.73	97.96	118.32
Neutral detergent fibre (g/kg)	99.94	89.10	69.15	56.04	42.98
Ca (g/kg)	11.64	11.36	10.85	10.51	10.18
Total Phosphorous (g/kg)	8.64	8.68	8.76	8.81	8.87
Na (g/kg)	1.13	1.26	1.51	1.67	1.83
K (g/kg)	9.56	9.89	10.50	10.90	11.31
Cu (mg/kg)	19.55	19.68	19.93	20.09	20.27
Mg (g/kg)	2.00	1.97	1.90	1.86	1.83
Mn (mg/kg)	139.0	135.2	128.3	123.7	119.2
Zn (mg/kg)	125.1	128.3	134.1	137.9	141.8
Indispensable amino acids					
Arginine (g/kg)	9.84	11.01	13.16	14.57	16.01
Histidine (g/kg)	3.56	4.03	4.90	5.48	6.06
Isoleucine (g/kg)	8.32	9.49	11.63	13.04	14.47
Leucine (g/kg)	13.59	15.43	18.83	21.06	23.32
Lysine (g/kg)	10.62	12.06	14.71	16.45	18.21
Methionine (g/kg)	3.14	3.59	4.41	4.96	5.51
Phenylalanine (g/kg)	8.98	10.04	11.99	13.27	14.56
Threonine (g/kg)	7.02	8.19	10.34	11.75	13.18
Valine (g/kg)	8.80	9.93	12.01	13.37	14.76
Dispensable					
Alanine (g/kg)	6.95	7.93	9.73	10.91	12.11
Aspartic acid (g/kg)	16.85	19.20	23.52	26.36	29.23
Glutamic acid (g/kg)	39.98	43.55	50.13	54.46	58.85
Glycine (g/kg)	5.96	6.84	8.47	9.55	10.63
Serine (g/kg)	6.01	6.88	8.49	9.55	10.62
Tyrosine (g/kg)	5.01	5.72	7.03	7.89	8.76

Table 14: Analysed composition of experimental diets for 8-12 weeks growth phase.

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
Dry matter (g/kg)	850.9	849.7	847.3	845.8	844.3
Crude protein (g/kg)	156.3	176.8	214.1	238.7	263.0
Gross energy (MJ/kg)	15.87	16.51	17.67	18.44	19.19
Ash (g/kg)	59.57	59.08	58.10	57.53	56.89
Crude fat (g/kg)	23.83	45.60	85.46	111.63	137.57
Ca (g/kg)	9.62	9.49	9.25	9.10	8.95
Total Phosphorous (g/kg)	7.98	7.88	7.68	7.56	7.44
Na (g/kg)	0.60	0.74	1.00	1.18	1.35
K (g/kg)	7.74	7.99	8.44	8.74	9.03
Cu (mg/kg)	16.08	16.50	17.24	17.75	18.23
Mg (g/kg)	1.96	1.91	1.81	1.75	1.69
Mn (mg/kg)	120.8	118.8	114.8	112.3	109.7
Zn (mg/kg)	124.3	128.5	136.0	141.1	146.0
Indispensable amino acids					
Arginine (g/kg)	6.73	7.93	10.11	11.55	12.97
Histidine (g/kg)	2.57	3.08	4.02	4.64	5.25
Isoleucine (g/kg)	5.96	7.18	9.41	10.89	12.34
Leucine (g/kg)	10.31	12.34	16.03	18.47	20.87
Lysine (g/kg)	8.60	9.78	11.92	13.33	14.73
Methionine (g/kg)	3.11	3.59	4.46	5.04	5.60
Phenylalanine (g/kg)	6.60	7.84	10.10	11.59	13.07
Threonine (g/kg)	4.77	5.94	8.06	9.46	10.85
Valine (g/kg)	6.83	7.89	9.82	11.09	12.35
Dispensable					
Alanine (g/kg)	5.17	6.06	7.68	8.75	9.80
Aspartic acid (g/kg)	11.52	14.08	18.76	21.84	24.89
Glutamic acid (g/kg)	30.74	34.65	41.77	46.47	51.10
Glycine (g/kg)	5.12	6.05	7.75	8.86	9.97
Serine (g/kg)	4.37	5.21	6.74	7.75	8.75
Tyrosine (g/kg)	3.53	4.26	5.58	6.45	7.31

Table 15: Analysed composition of experimental diets for 12-16 weeks growth phase.

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.3	849.8	850.6	851.2	851.7
Crude protein (g/kg)	138.1	156.8	191.1	213.6	236.3
Gross energy (MJ/kg)	15.75	16.38	17.51	18.25	19.01
Ash (g/kg)	51.45	51.87	52.58	53.01	53.51
Crude fat (g/kg)	20.12	40.87	79.13	104.2	129.5
Ca (g/kg)	8.66	8.75	8.91	9.01	9.12
Total Phosphorous (g/kg)	7.37	7.39	7.43	7.45	7.48
Na (g/kg)	0.68	0.76	0.91	1.01	1.11
K (g/kg)	6.79	6.93	7.18	7.33	7.50
Cu (mg/kg)	18.08	19.49	22.08	23.76	25.47
Mg (g/kg)	1.70	1.64	1.52	1.44	1.36
Mn (mg/kg)	124.8	126.6	129.7	131.7	133.8
Zn (mg/kg)	114.6	116.7	120.4	122.8	125.2
Indispensable amino acids					
Arginine (g/kg)	5.90	6.92	8.79	10.01	11.25
Histidine (g/kg)	2.42	2.85	3.64	4.16	4.69
Isoleucine (g/kg)	5.31	6.28	8.05	9.21	10.38
Leucine (g/kg)	9.20	10.66	13.35	15.10	16.88
Lysine (g/kg)	8.57	9.68	11.73	13.08	14.43
Methionine (g/kg)	3.89	4.44	5.44	6.10	6.76
Phenylalanine (g/kg)	6.16	7.01	8.58	9.61	10.65
Threonine (g/kg)	4.56	5.58	7.47	8.70	9.95
Valine (g/kg)	6.65	7.62	9.41	10.58	11.77
Dispensable					
Alanine (g/kg)	4.71	5.53	7.04	8.03	9.03
Aspartic acid (g/kg)	9.64	11.62	15.27	17.66	20.07
Glutamic acid (g/kg)	32.21	35.43	41.34	45.20	49.12
Glycine (g/kg)	4.80	5.72	7.41	8.52	9.64
Serine (g/kg)	3.98	4.73	6.10	7.00	7.91
Tyrosine (g/kg)	2.90	3.41	4.36	4.99	5.61

Table 16: Analysed composition of experimental diets for 16-20 weeks growth phase.

Determined values	Crude protein and energy concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	849.7	851.3	854.2	856.2	858.1
Crude protein (g/kg)	120.0	133.7	159.3	176.1	193.1
Gross energy (MJ/kg)	15.77	16.42	17.64	18.45	19.27
Ash (g/kg)	46.41	45.85	44.88	44.23	43.59
Crude fat (g/kg)	20.06	44.73	90.44	120.65	151.01
Ca (g/kg)	8.50	8.40	8.22	8.10	7.98
Total Phosphorous (g/kg)	6.72	6.79	6.91	7.00	7.08
Na (g/kg)	0.77	0.83	0.95	1.03	1.12
K (g/kg)	6.04	6.04	6.06	6.08	6.09
Cu (mg/kg)	17.68	17.28	16.56	16.09	15.62
Mg (g/kg)	1.62	1.54	1.39	1.30	1.20
Mn (mg/kg)	123.3	121.9	119.7	118.2	116.7
Zn (mg/kg)	122.4	124.8	129.4	132.5	135.6
Indispensable amino acids					
Arginine (g/kg)	4.65	5.32	6.58	7.41	8.25
Histidine (g/kg)	2.04	2.27	2.70	2.99	3.28
Isoleucine (g/kg)	4.30	5.10	6.59	7.57	8.55
Leucine (g/kg)	7.76	8.95	11.15	12.61	14.07
Lysine (g/kg)	5.96	6.59	7.77	8.55	9.34
Methionine (g/kg)	1.92	2.40	3.29	3.88	4.47
Phenylalanine (g/kg)	5.29	5.98	7.26	8.11	8.97
Threonine (g/kg)	2.55	3.12	4.19	4.89	5.60
Valine (g/kg)	5.12	5.91	7.38	8.35	9.33
Dispensable					
Alanine (g/kg)	3.74	4.30	5.33	6.01	6.70
Aspartic acid (g/kg)	7.34	8.92	11.87	13.81	15.77
Glutamic acid (g/kg)	29.39	31.68	35.94	38.76	41.60
Glycine (g/kg)	4.15	4.89	6.27	7.18	8.09
Serine (g/kg)	3.21	3.66	4.51	5.06	5.62
Tyrosine (g/kg)	2.08	2.50	3.26	3.77	4.28

3.2.3 Comparison of turkeys growth performance

One hundred and eighty five day old male turkeys (BUT 8) were weighed and placed in a controlled environment building. For the pre-study period (first 4 weeks of age) birds were placed in the floor pen containing 10 cm thick bedding material of wood shaving. During the pre-study period all birds were offered the same standard turkey starter crumb diet and had *ad libitum* access to feed and water. Birds were wing tagged at day 10 for identification. The average air temperature of the house was recorded every day and was maintained at 30°C for 7 days and gradually reduced to 22°C at 4 weeks of age. For the first day 24 hour light was provided which than changed to a lighting schedule of 16 hour light and 8 hour dark period through out the trial.

At twenty-eight days of age one hundred and seventy five turkeys were transferred to 35 floor pens, using stratified randomisation on body weight, 5 birds in a pen (1.01 x 0.35 m/pen floor area) within a controlled environment room. All the pens were equipped with plastic feed hoppers and drinkers. The experiment was a randomized block design consist 5 treatments (5 levels of CP and ME concentrations and 4 feeding/ growth phases) each dietary treatment was replicated 7 times with 5 birds in each replicate. Feed and water were offered *ad libitum* throughout the experiment. The whole experimental period of 16 weeks starting from 4 weeks of age was divided into 4 weeks standard growth phases: 4-8, 8-12, 12-16 and 16-20 weeks, finish at 20 weeks of turkey's age, according to commercial management guide for BUT 8 (Aviagen Turkeys Ltd.). The same house environment as for the end of the pre-study period was provided until the end of the study. The experiment ended when the birds were 20 weeks of age.

3.2.4 Water intake

A plastic header tank with a recorded weight of water was placed on the corner of each pen (Figure 4) for water intake determination each week for a period of 24h. On the day of water intake determination a turkey bell drinker was attached to the header tank and after 24h the water intake was recorded as the difference between the water offered and the water remained in the header tank at both occasions. To get the measurements of evaporative losses five bell drinker with identical volume of water were placed each day at bird height and at different points within the experimental room but out of the reach of birds. The water measurements then were recorded as kg/bird/day after correcting the evaporative losses.

Figure 4: Arrangement for water intake measurements.

3.2.5 Feed intake

To determine the feed intake, the feed offered at the beginning of each growth phase was recorded and the weigh back was done at the end of each phase. During the digestibility trial (on 49th day of the trial), feed intake was determined separately to get the feed intake for 24h. The values of daily feed intake were recorded in kg/day/bird.

3.2.6 Body weight (BW)

Birds were weighed individually before placing them in pens to get the initial weight and then on a 4 weekly basis birds in each pen were weighed individually to get the measurements for body weight gain. This was then converted to body weight gain in kg/day/bird.

3.2.7 Excreta collection

For the determination of dietary nutrient digestibility coefficients (i.e. DM, CP, amino acids, minerals, organic matter, ash and metabolisable energy) excreta was collected for a

period of 24h at 7 weeks of age. Excreta were freeze-dried, weighed and milled to pass through a 0.75mm mesh.

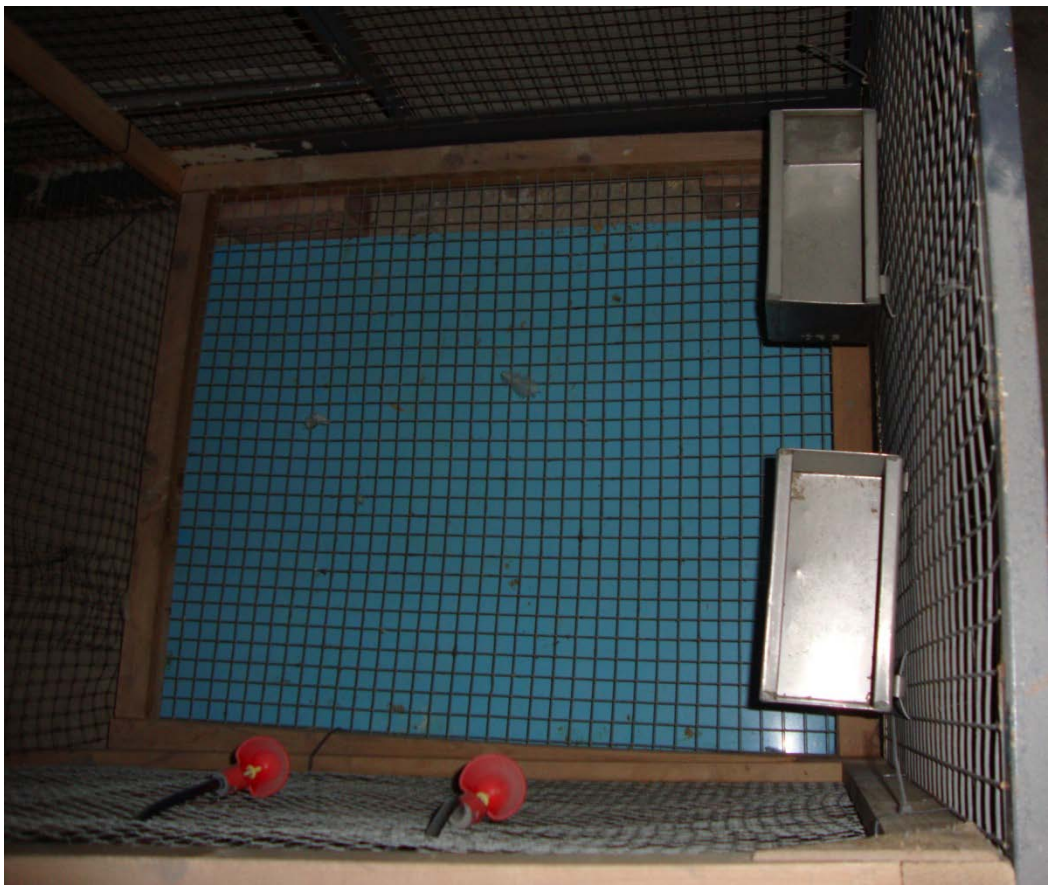


Figure 5: Raised floor pen arrangement for excreta collection.

3.2.8 Footpad dermatitis (FPD) scoring

Footpad lesions were scored for the left and right foot and classified according to a scale from Hocking *et al*. (2008) from 0 (no lesion) to 4 (very severe lesions) for FPD and all animals were scored at the end of week 8, 12, 16 and 20. FPD scoring was done on bird weighing days.

Score 0

Score 1

Score 2

Score 3

Score 4

(Hocking *et al.*, 2008)

Figure 6: Footpad dermatitis scoring guide.

0 = no external signs of FPD. The skin of the footpad feels soft to the touch and no swelling or necrosis was evident.

1 = the pad feels harder and denser than a non affected foot. The central part of the pad was raised, reticulate scales were separated and small black necrotic areas may be present.

2 = marked swelling of the footpad. Reticulate scales were black, forming scale shaped necrotic areas. The scales around the outside of the black areas may have turned white. The area of necrosis was less than one quarter of the total area of the footpad.

3 = swelling was evident and the total footpad size was enlarged. Reticulate scales were pronounced, increased in number and separated from each other. The amount of necrosis extended to one half of the footpad.

4 = as score 3, but with more than half the footpad covered by necrotic cells.

The score was recorded on the FPD data captured form (see example attached).

Replicate	Total birds	Birds tag number	Number of birds in category				
			Score=0	Score=1	Score=2	Score=3	Score=4

Table 17: Footpad dermatitis score capturing form.

The footpad score (FPS) i.e. total FPS, good FPS and bad FPS were calculated for each pen by using following equations, e.g. for 10 birds in a pen had different scores i.e. 1 bird score 1, 2 birds scored 2, 3 birds score 3 and 4 birds had score 4 the total FPS score for that pen will be 3. Whereas, for GHS if all the birds had some lesions then that pen will have 0 GHS, and bad hock score will be 1.

The total footpad scores (TFPS) were calculated for each pen as follows:
[(0 x n) + (1x n) + (2 x n) + (3 x n) + (4 x n)] / Total number of birds scored.

Where the number from 0 to 4 was the score as described and “n” was the number of birds corresponding to each score in the pen. A lower score will be associated with better leg health.

The good footpad scores (GFPS) were calculated for each pen as follows:
[n 0 / Total number of birds scored]

Where “n 0” is the number of birds with score 0 (without problems) in the pen. A greater score for GFP will be associated with better leg health.

The bad footpad scores (BFPS) were calculated for each pen as follows:
[(n 1 + n 2 + n 3 + n 4) / Total number of birds scored]

Where “n 1”, “n 2”, “n 3 and “n 4” is the number of birds with score 1, 2, 3 and 4, respectively, in the pen. A lower score for BFP will be associated with better leg health. It is expected that sum of the good and the bad scores should be 1.

Equation 14: Equations for the calculations of total FPS, good FPS and bad FPS per pen.

Following images of footpad dermatitis scores were taken from the live birds whereas, the images used as reference were from birds after slaughtering.

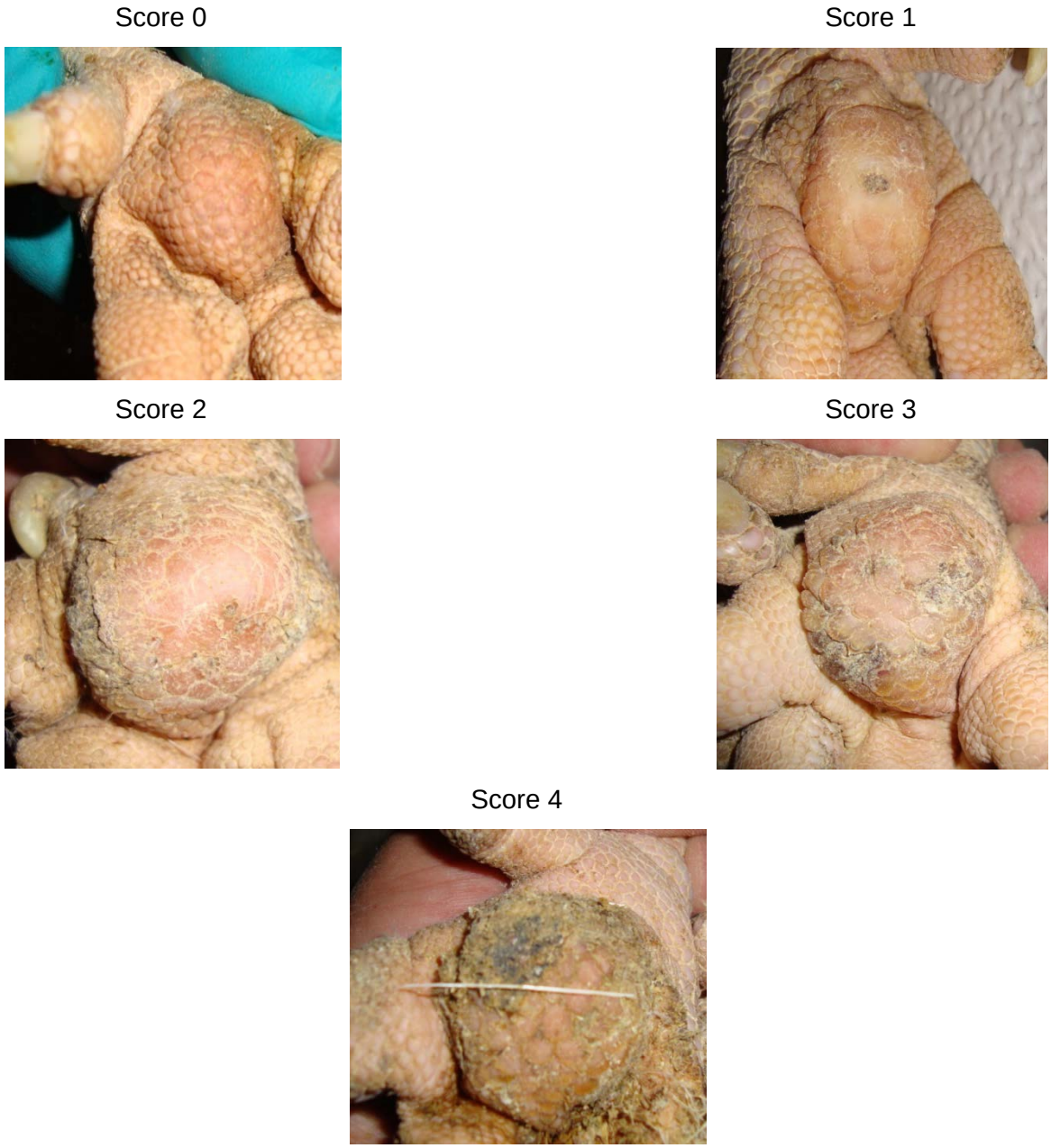


Figure 7: Footpad dermatitis images from live birds.

3.2.9 Hock burn (HB) scoring

Hock lesions were scored for both the left and right leg and classified according to a scale from 0 (no lesion) to 4 (very severe lesions). All animals were scored at the end of week 8, 12, 16 and 20. The operator undertaking the hock score assigned one of five scores, using the following guide.

Figure 8: Hock burn score (HBS) guide for turkeys.

0 = no discolouration/burning/scalding

1 = slight discolouration and bruised appearance of skin in hock area

2 = discolouration/scabs/black necrotic tissue on or between scales (total area up to 0.5cm diameter)

3 = as score 2 but well established scab/burnt areas, lesion area covers up to a third of the hock

4 = hock enlarged with large scab/burnt area, as score 3 but lesion area covers more than a third of the hock area

This scale was devised by me as no other suitable scale existed. The scores were recorded on the hock scoring data capture form (See example attached).

Replicate	Total birds	Birds tag number	Number of birds in category				
			Score=0	Score=1	Score=2	Score=3	Score=4

Table 18: Hock burn score capturing form.

The hock score (HS) i.e. total HS (THS), good HS (GHS) and bad HS (BHS) was calculated for each pen by using following equations.

The total hock burn scores (THS) were calculated for each pen as follows:
[(0 x n) + (1x n) + (2 x n) + (3 x n) + (4 x n)] / Total number of birds scored.

Where the number from 0 to 4 was the score as described and “n” was the number of birds corresponding to each score in the pen. A lower score will be associated with better leg health.

The good hock scores (GH) were calculated for each pen as follows:
[n0 / Total number of birds scored]

Where “n 0” is the number of birds with score 0 (without problems) in the pen. A greater score for GH will be associated with better leg health.

The bad hock scores (BH) were calculated for each pen as follows:
[(n 1 + n 2 + n 3 + n 4) / Total number of birds scored]

Where “n 1”, “n 2”, “n 3 and “n 4” is the number of birds with score 1, 2, 3 and 4, respectively, in the pen. A lower score for BH will be associated with better leg health. It is expected that sum of the good and the bad scores should be 1.

Equation 15: Equations for the calculations of total HBS, good HBS and bad HBS per pen.

3.2.10 Litter scoring

A visual assessment of the entire pen was done at the end of each growth phase, using the following images and guideline details e.g. the total area of the pen was scored by attributing a percentage value to the litter which scored 1 to 5. For example if 50% of the area scored to 1 and 30% to 2, and 20 % scored 3 then the equation used would be:

[(1x 50%) + (2 x 30%) + (3 x 20%) + (4 x 0%) + (5 x 0%)]/100: Score = 1.7. A lower score will be associated with better litter quality.

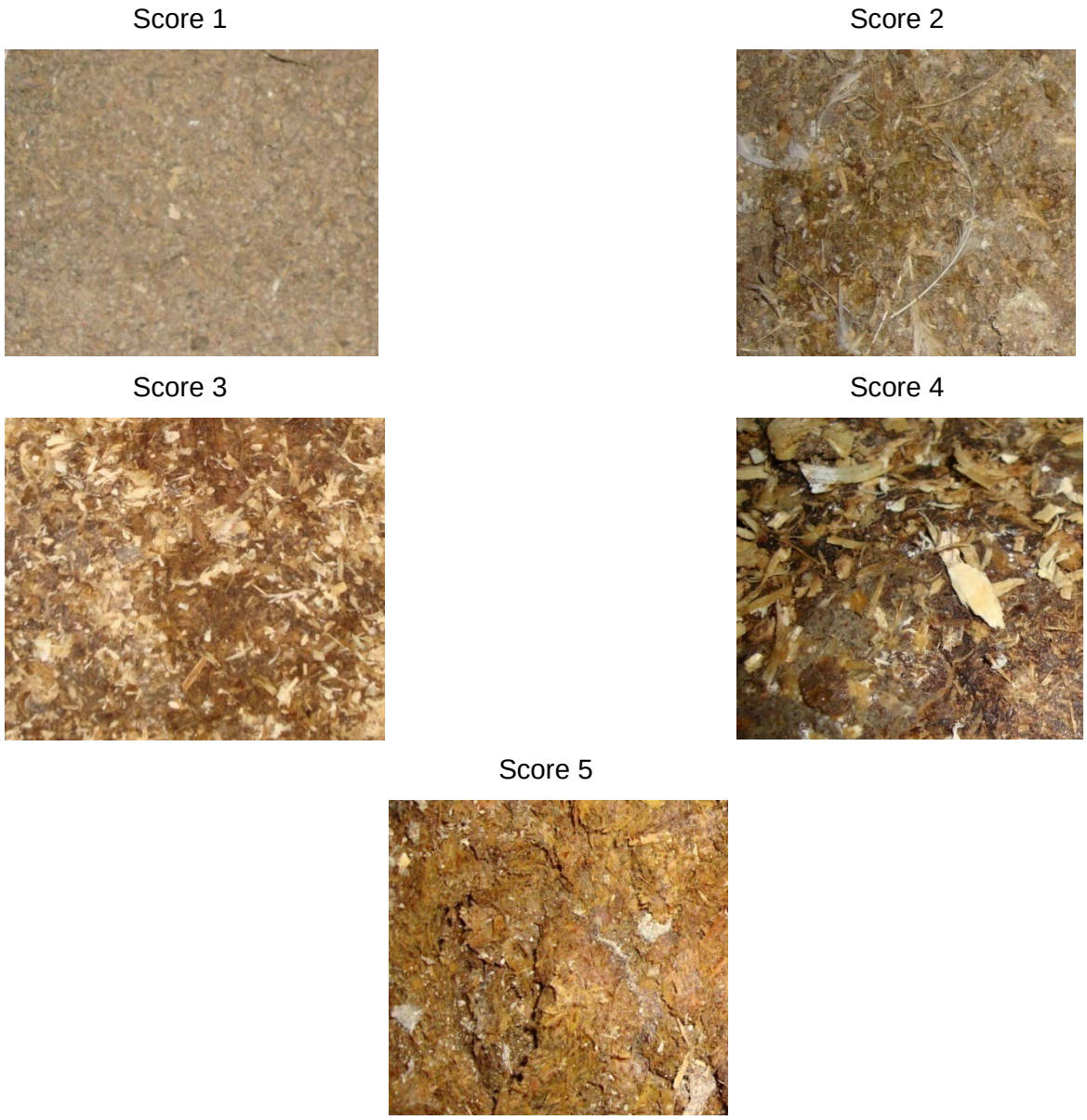


Figure 9: Litter scoring guide.

1 = friable, no capping or compaction whatsoever

2 = light capping, under a friable crumb surface

3 = surface capped and compacted

4 = surface wet and sticky

5 = litter depth wet and dough-like

A percentage of each pen was decided, to the nearest 5%, in each score category. Litter score were calculated and recorded as follows:

[(1 x %) + (2 x %) + (3 x %) + (4 x %) + (5 x %)] / 100

Equation 16: Equation to calculate litter score for each pen.

The average litter score was calculated and recorded on the data record form.

Replicate	Total birds	Percentage of each pen, to the nearest 5%, in each score category				
		Score=1	Score=2	Score=3	Score=4	Score=5

Table 19: Data capturing form for Litter score.

3.2.11 Litter pH and atmospheric NH_3 determination

The litter pH was determined at 4 weekly intervals from 4 to 20 weeks of age by using the pH probe directly in to the litter and in the centre of each pen. Litter pH was determined by using a stab pH probe (pH probe with stainless steel penetration blade) attached to a Hanna HI 99163 meter (Hanna Instruments Ltd., Bedfordshire, UK).

Atmospheric ammonia was measured using a handheld Dräger meter tube (Ammonia 2/a) attached to a Dräger Multi Gas Detector pump (Draeger Safety AG and Co. KGaA, Luebeck, Germany) (Figure 10). Ammonia concentrations were recorded from each pen, almost 3 cm above litter surface and from the central point of the pen by stroking the pump five times (approximate one minute/pen). The Dräger tubes change from yellow to blue for a positive value for ammonia. The principle of the reaction is:
NH? + pH indicator → blue reaction product.

Readings of the pH and NH_3 meters were recorded on the data capturing form. The standardisation of the pH probe was carried out according to the SOP adopted in SAC Ayr.

Figure 10: Apparatus (dragger pump and tube) to determine ammonia concentration at bird level.

Figure 11: Apparatus (pH meter and probe) to determine litter pH.

3.2.12 Litter analysis

Litter samples were taken from the centre and mid way between centre and four corners of each pen at the end of each growth phase. The litter samples collected were combined

and homogenized in plastic bags and the moisture contents were determined by placing in an oven at 80°C for 48 hours and using Equation 5 Section 2.2.3.

3.2.13 Dietary nutrient digestibility and AME determination

To determine dietary nutrient digestibility and AME at 7 weeks of age, all the birds from each pen were transferred to one of the 35 raised floor cages for 24 hours (Figure 5). The excreta voided were collected on trays placed beneath each cage and the feed intake for the same period was determined. Then excreta samples were freeze dried, weighed and milled to pass through a 0.75 mm mesh.

The methodology for the determination of apparent Metabolisable Energy and nutrient digestibility are described in Section 2.2.4.

3.2.13.1 **Amino acid determination (HPLC)**

The amino acid content of feed and excreta was determined by High performance liquid chromatography following oxygen-free hydrochloric acid digestion (Jones *et al.*, 1981). The system comprised a Dionex ASI-100 autosampler fitted with a Dionex P580 pump and a Dionex RF-2000 detector (Sunnyrale, California, USA). The flow rate used was 1 mL min^{-1} and the column used was a Spherisorb ODS2 (150x4.6mm fitted with a Waters guard cartridge). Since this method of hydrolysis destroys methionine, cystine and tryptophan, data on these amino acids are not reported. Metabolisability coefficient for glycine is not presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al.*, 1971).

Simplified method for amino-acid analysis

1. Weigh 50 – 100 mg sample into a screw capped glass hydrolysis tube.
2. About the same weight for casein – 2 samples as a standard per 40 done.
3. Add 5ml Hydrochloric acid, 6N [A] to the tube.
4. Place in an ultrasonic bath for 15 minutes in order to mix the contents.
5. Flush the tubes with oxygen free Nitrogen for 30 seconds and seal.
6. Hydrolyse for 24 hours by placing tube in a heating block previously heated to 110°C ± 1°C. Check after 1-2 h in oven for loose caps and retighten.
7. After hydrolysis, remove the tube from the heating block, cool to room temperature.
8. Transfer the contents to a 50 ml volumetric flask and dilute to volume with water.
9. Filter the hydrolysate through Whatman No. 4 paper (or equivalent) into a 50 ml polythene bottle. Stable for at least 8 weeks at room temperature.

10. Place 1 – 10 ml see table below (Excreta usually 5 ml; Feed - 2.5 ml and Casein 1.0 ml) and 0.5 ml internal standard solution [C] into a 50ml Quick fit round bottom flask and dry at 65 °C under vacuum. Reduce temperature and/or vacuum pressure if sample starts to bubble. Try between 40-50 °C.
11. Dissolve the residue in 2.5 ml Acetic acid, 25mM [B] and transfer to 20ml polythene.
12. Working standard - Place 0.5 ml standard mixture [D] and 0.5 ml internal standard [C] in a 50 ml round bottomed flask. Evaporate to dryness at 40°C under vacuum Dissolve residue in 2.5 ml acetic acid, 25 mM [B]. Final concentration is 0.5 µmoles per ml for all components. Stable for at least one month if stored at 0-5°C.

Protein content	0-15%	15-30%	30-50%	>50%
Drying vaolume	10.00 ml	5.00 ml	2.50 ml	1.00 ml
		Excreta	Feed	Casein

Stock Solutions

A. 6N HCL – add with stirring 516 ml concentrated hydrochloric acid to about 400ml water, cool and dilute to volume with water in a 1000ml volumetric flask (In fume chamber).
B. 25 mM Acetic Acid - add 1.45 ml glacial acetic acid to about 500ml water, dilute to volume with water in a 1000ml volumetric flask (In fume chamber).
C. Internal standard (2.5 µmoles per ml) - dissolve 25.78 mg dl-α-amino-n-butyric acid in about 50 ml water, dilute to volume with water in a 100 ml volumetric flask. Store at 0-5 °C.
D. Amino acid standard mixture (2.5 µmoles per ml) - Purchased from Sigma Chemicals (Cat. No. AA-S-18). Store at 0-5 °C.

Figure 12: HPLC apparatus used to determine amino acid concentration in feed and excreta.

Images of chromatography for excreta, feed, casein and standard are given below to indicate the time and peaks of different amino acids.

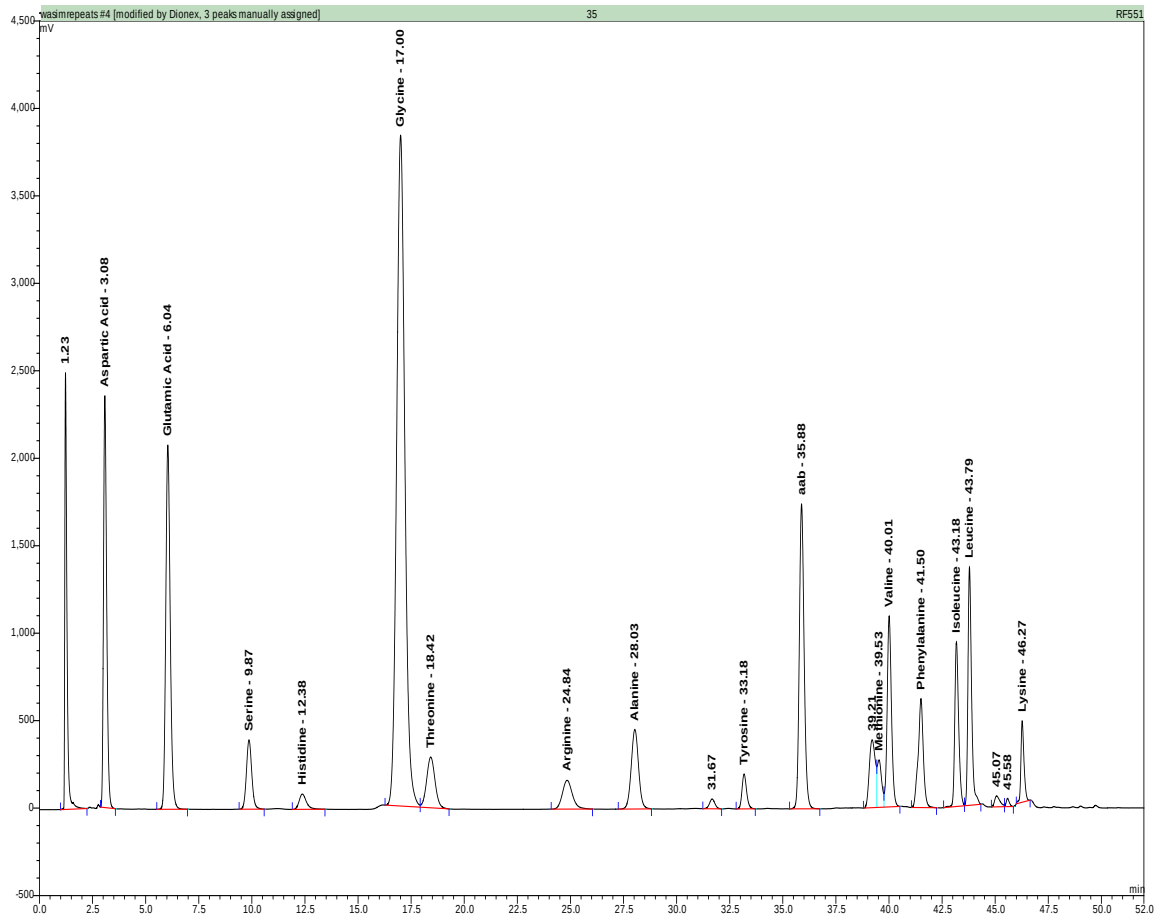


Figure 13: Chromatography image for amino acids peaks and time for excreta sample.

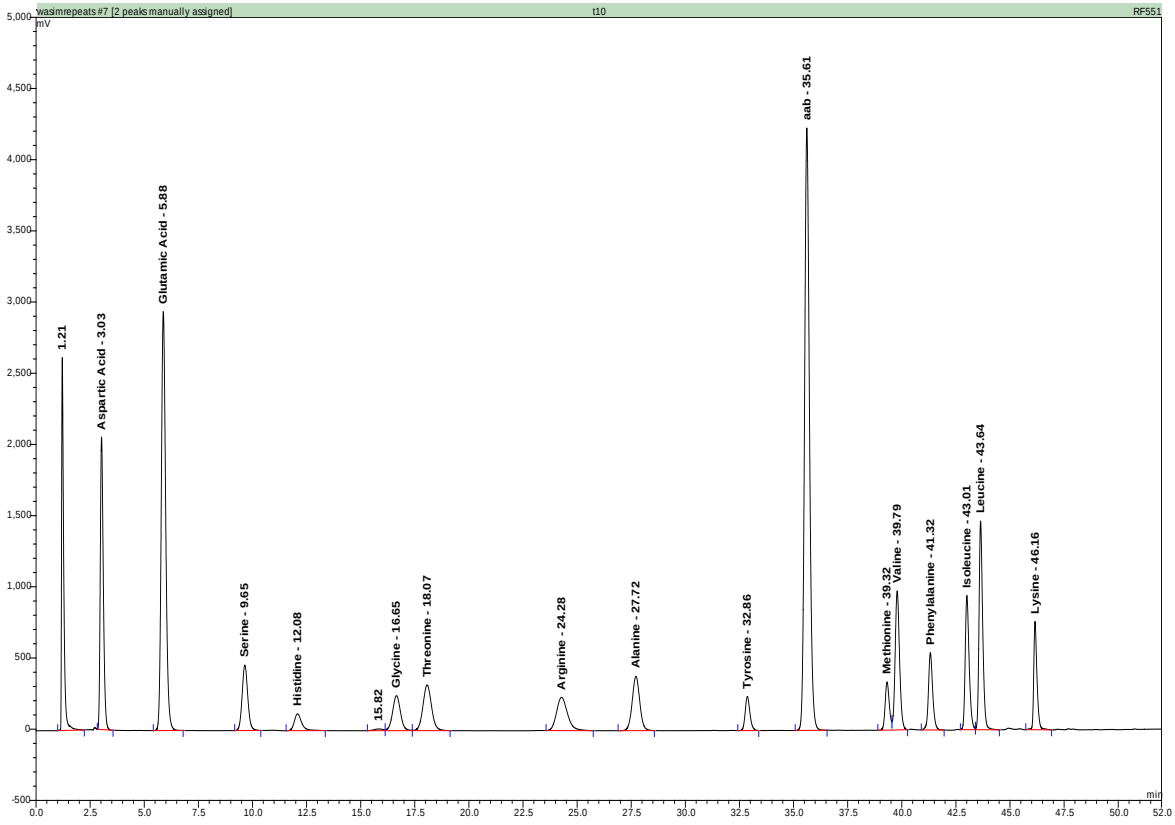


Figure 14: Chromatography image for amino acids peaks and time for feed sample.

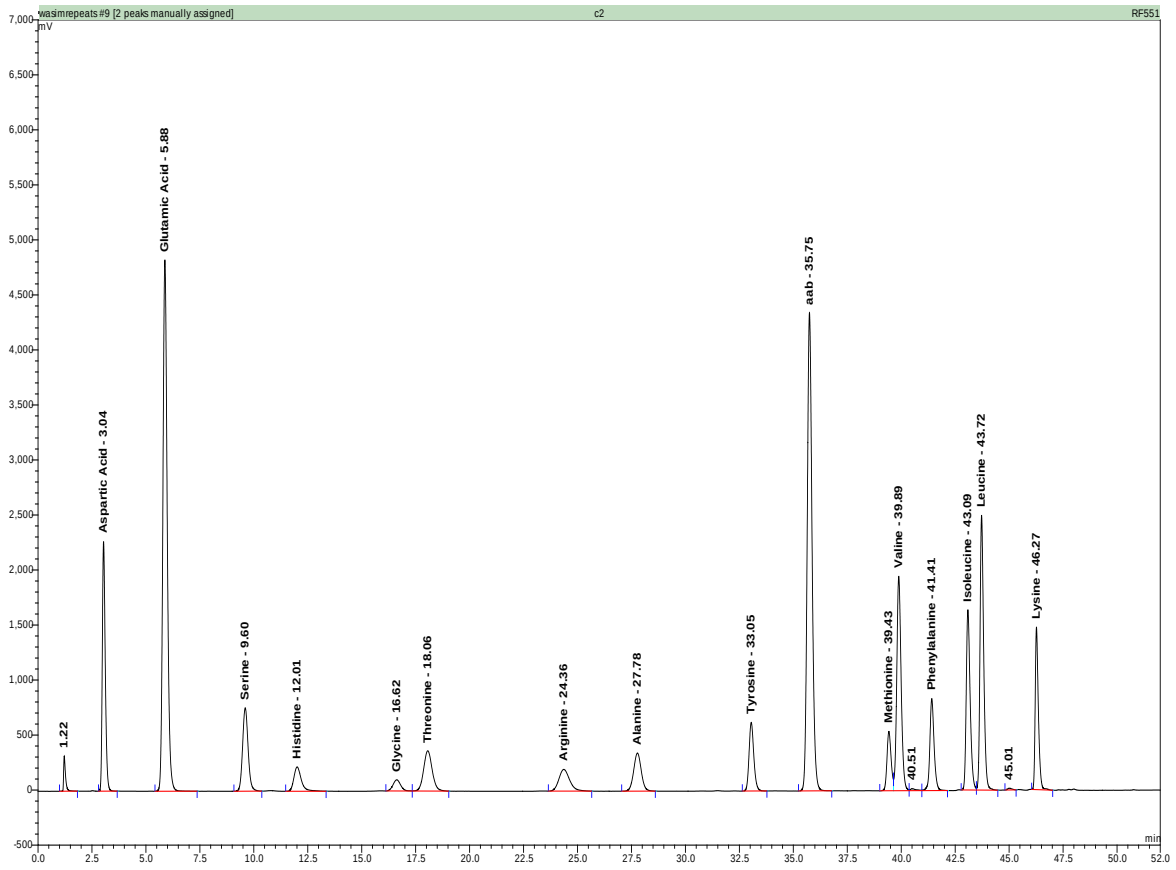


Figure 15: Chromatography image for amino acids peaks and time for casein sample.

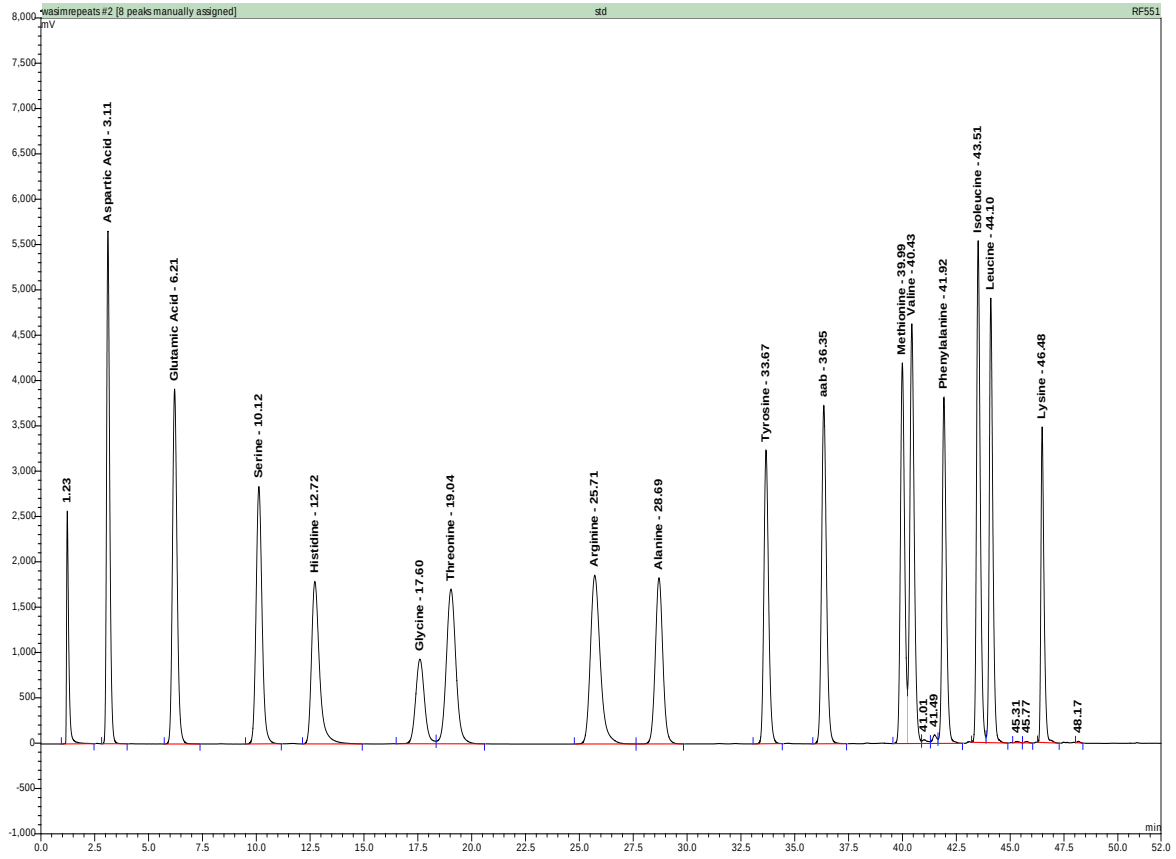


Figure 16: Chromatography image for amino acids peaks and time for standard.

3.2.13.2 **Mineral determination**

The procedure followed for mineral analyses (Na, Ca, P, K, Mg, Zn and Mn) in samples of feed and excreta was the same; the digestion of samples was carried out by using Microwave Accelerated Reaction System (MARS) as used for the rapid preparation of sample for atomic absorption and the optical plasma emission spectrometry (Optima 4300 DV Dual View ICPOE spectrometer, Perkin Elmer, Beaconsfield, UK), (Tanner *et al.*, 2002). The MARS uses microwave energy to heat samples. A sample placed inside a microwave vessel with acid is subjected to rapid heating and elevated pressures, causing the sample to digest in a short time.

3.2.14 Statistical procedure

Seven replicates per treatment were used for the experiment with a total of one hundred and seventy five turkeys. For the analysis of data, statistical measurements, average, and standard errors of differences of means were obtained for all numeric variables analyzed (descriptive statistical techniques). Randomised complete block analysis of variance (ANOVA) model, with two factors (treatment and time) for repeated measures, including the Greenhouse–Geiser degrees of freedom corrections and ANOVA for two factors, when the analysis was performed between treatments and times (inferential statistical techniques) (Zar, 1999). The model included dietary nutrient density (5 levels of dietary nutrient concentration), time (weeks ending the growth phase i.e. 8, 12, 16 and 20), and the interaction between dietary density and weeks ending the growth phases. The pens were treated as experimental units. Orthogonal polynomials were also used for average values of all numeric variables (e.g. litter moisture, litter NH_3, litter pH etc.) to compare treatment differences for linear and quadratic relationships with increasing dietary nutrient concentration. Comparison contrast test was used on the average values of all numeric variables analyzed (above mentioned) to compare low nutrient density diets (i.e. 77 and 85% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation) as well as high nutrient density diets (i.e. 110 and 120% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation).

However, for data i.e. AME, AMEn, AME I, CP D, DM D, OMI, OMEx, OMD, EER, NEx, AAN, UAN, NDF I, NSC I, Ash digestibility and amino acid intake, excretion, retention and digestibility values determined after 7^{th} weeks of birds age (at 49^{th} day of birds age) a randomized complete block analysis of variance was performed to compare the main treatment effect (5 levels of dietary nutrient concentration, crude protein and energy). An orthogonal polynomial contrast test was used to quantitatively compare the linear and

quadratic regression effects. The data entered on an Excel spreadsheet and Genstat software, release 11 (IACR Rothamstead, Harpenden, Hertfordshire) was used to perform ANOVA for the comparison of different treatments for litter quality parameters i.e. moisture, NH_3, pH and temperature and other parameters such as water intake, feed intake, body weight gain, feed conversion efficiency and nutrient digestibility. Comparison contrast test was used to compare low nutrient density diets (i.e. 77 and 85% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation) as well as high nutrient density diets (i.e. 110 and 120% of standard breed recommendation) and standard nutrient density diet (100% of standard breed recommendation). Correlation coefficients were also generated on average values to test for a possible relationship between different variables. Differences were reported as significant at $P<0.05$ and trends were noted when the P value was near to 0.1.

The data obtained for FPS and HBS were compared using the values (weighted means for each pen for TFPS and THS) explained in Sections 3.2.8 and 3.2.9, for each pen for GHS, BHS, THS scores and for GFPS, BFPS and TFPS scores, by using ANOVA for the comparison of different treatments. There were not enough different non zero scores to make a multinomial analyses (or chi-squared) possible for FPS and HBS data (real values) and also, it was not possible to incorporate the random structure in the data using Chi-squared, however, since the residual plot were unacceptable after running residual maximum likelihood (REML). Therefore, generalized linear mixed models (GLMM), were fitted using residual maximum likelihood (REML) to binary data: FPD>0, or not, and HB>0, or not (binomial, link logit transformed) and fixed effects time+treatment and random effects bird weight category, block and pen with dispersion fixed at 1. There was not enough information in the data to include the interaction term (i.e. time x treatment). The P-values, estimated means, SEMs and back transformed means are reported in the result tables. Since no FP lesions appeared at the end of week 8 the data for FPS, this time point was not included in analysis.

3.3 **Results**

The birds remained healthy and overall mortality was less than 1% throughout the experiment, with no significant difference between treatment groups (data not shown).

The Analysed chemical composition of the basal diets is presented in tables (Table 13 to Table 16). The analysed values for the concentration of CP content were lower than the calculated values in Table 9 to Table 12, however, the analysed values for K, Ca and Na concentration were higher than the calculated values. Digestible amino acid data taken from the literature was derived from studies on the birds of varying breed, sex and age as

well as method of digestibility determination (ileal and total tract). In contrast the data collected during the course of this study has been obtained from controlled groups of birds of same breed, sex and age as well as using total tract method for digestibility determination, so no comparison is made here.

3.3.1 Water intake measurements

Increased nutrient density had a negative effect on water intake (WI) and feed intake used for water:feed determination (feed intake measured for 24h time period to determine water:feed, FI W:F) which decreased linearly (P<0.01 and 0.001, respectively) as the density increased (Table 25). However there was no effect (P>0.05) of the dietary nutrient density recorded on water:feed (W:F). The WI, FI W:F linearly increased (P<0.001) with the increase of the age of the birds, the WI (Figure 27) and FI W:F values were observed during the last feeding phase of the study. The increase of the birds age had a negative effect (P<0.01) on W:F and the lowest values were recorded in the last two feeding phases of the study (Table 25). The results for WI, FI W:F and W:F were subject to a dietary density x time interaction (P<0.001 for WI and P<0.05 for the rest), showing that the responses to feed density were different during growing periods (Table 25). For example, an increase in nutrient density during the first feeding phase led to an increase in WI, although the response during the rest of the feeding phases was the opposite and the WI decreased when nutrient density increased (Figure 28). An increase in dietary density did not have significant effect on the FI W:F during the first two feeding phases, but led to a decrease FI during the last two feeding phases. Dietary density increased W:F during the first feeding phase, although the responses of W:F were inconsistent for the rest of the study (Table 25).

3.3.2 Litter quality associated parameters

Increased nutrient density had a negative effect on litter moisture (LM), and litter score (LS) which decreased in a linear way (P<0.01 and 0.001, respectively) as the density increased (Table 20). However, the LM and LS linearly increased (P<0.001) with the increase of the age of the birds, the highest LM (Figure 21) and LS (Figure 25) values were observed during the last feeding phases of the study (Figure 22 and Figure 26). Increased nutrient density had a positive effect on litter ammonia (NH_3) which increased in a linear way (P<0.001) as the density increased (Table 20). The time response of litter NH_3 concentration was also quadratic (P<0.01) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases (Figure 24). Litter pH tended (P=0.06) to have a quadratic response to dietary density (Table 20). The time response of litter pH was also quadratic (P<0.001) as the highest values were observed

for the second (8-12 week) and third (12-16 week) growing phases (Table 20). Litter temperature (T°) was not affected by dietary density (P>0.05) but responded in a quadratic manner to time as the lowest T° was observed between 8-12 weeks of age. The results for litter ammonia and litter score (NH_3 and LS, respectively) were subject to a dietary density x time interaction (P<0.05), showing that there were different patterns of response during different growing phases. For example, the response of the LS to diets T4 and T5 seems not to be influenced by the feeding phase (Figure 26) although the response of feeding the rest of the diets tended to follow a quadratic pattern (Table 20). The response of litter NH_3 to dietary density during different feeding phases was also inconsistent (Figure 24). The comparison contrast test did not find a difference in LM, pH, T° and LS between diet T3 and low nutrient density group (T1 and T2) as well as diet T3 and higher nutrient density group (T4 and T5). However, significantly higher litter NH_3 was recorded in groups fed the control diet when compared with groups fed lower nutrient density diets (Table 20), whereas, no difference (P>0.05) was recorded when the control diet fed group was compared with higher nutrient density fed groups.

3.3.3 Leg health parameters

As nutrient density increased so did the prevalence of hock burn (P<0.05). Increasing nutrient density had a negative linear effect (P<0.05) on good hock scores (GHS). It, however, resulted in a linear increase in bad hock scores (BHS) and total hock scores (THS) (P<0.05 and P<0.01, respectively) (Table 21). The growth phases had significant effect (P<0.001) on all hock score parameters, where GHS increased with growth phases, conversely BHS and THS (Figure 17 and Figure 18) decreased as the bird aged. There was no time and diets interaction noted (P>0.05) for hock burn parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05) (Table 21). There was no effect of nutrient densities observed (P>0.05) for the footpad quality score (Table 22). However, growth phase had a significant effect (P<0.001) on all foot score parameters, where good footpad scores (GFPS) increased with growth phases, conversely bad footpad scores (BFPS) (Table 22) and total footpad scores (TFPS) (Figure 19 and Figure 20) decreased (P<0.001) as the birds aged. There was no time by diets interaction noted (P>0.05) for footpad quality parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05) (Table 22).

As for hock burn (HB) the results obtained showed an increase in HB incidence in birds fed diet containing higher nutrient density (P<0.05). However, there was a significant decrease (P<0.001) in the incidence of HB as birds grew older 56% vs. 16% birds with HB>0 at the end of week 8 and 20, respectively (Table 23). The incidence of footpad

dermatitis (FPD) however, was not affected by treatment (P>0.05). However, the effect of time period was significant (P<0.001) for both HB and FPD as there were higher incidences recorded at the end of weeks 8 and 12, respectively which fell at the end of week 16 with an increase at week 20 (Table 22).

Correlations between variables are shown in (Table 28). Hock burn score (HBS) was associated with many of the parameters and in particular water to feed ratio (r = 0.930; P<0.001), feed conversion efficiency (r = 0.922; P<0.001), water intake (r = -0.906; P<0.001) and ammonia in litter (r = 0.813; P<0.001). Interestingly, footpad score (FPS) was only associated with the water to feed ratio (r = - 0.663; P<0.001).

3.3.4 Growth performance, dietary nutrient intake and utilisation

Overall body weight (BW) was higher than the breed standards at 20 weeks of age, i.e. 18.81 kg vs. target of 15.18 kg (data not included in tables). Increased nutrient density had a positive effect on total weight gain (TWG), weight gain (WG) and feed conversion efficiency (FCE) which increased following a linear pattern (P<0.001) when density increased (Table 24). Increasing nutrient density had a negative linear effect (P<0.001) on feed intake (FI). TWG and WG increase (P<0.001) with the increase in the age of the birds whereas FCE decreased linearly (P<0.001) with the increase in the age of the birds. The protein efficiency ratio (PER) response to feed density was also linear (P<0.05) and as expected, the PER decreased (P<0.001) with age. The FCE value for the control diet was higher (P<0.001) than the lower nutrient density fed group, and lower (P<0.001) than the higher nutrient density fed group, respectively (Table 24). The results for TWG, WG and FI were subject to a dietary density x time interaction (P<0.001), showing that the responses to feed density differed with age. The response of TWG and WG to nutrient density was linear (P<0.001) during the growth phases consist of 4-8 and 8-12 weeks. While a non-significant (P>0.05) effect of dietary nutrient density on these parameters were recorded during 12-16 weeks time period, whereas, the response of these parameters to dietary nutrient density was quadratic (P<0.05) during time period 16-20 weeks. The response of FI to nutrient density was linear (P<0.001) during growth phases consisting of 4-8, 8-12 and 12-16 weeks. Whereas, the response of FI to dietary nutrient density was quadratic (P<0.05) from 16-20 weeks.

Nutrient density had a positive and linear effect (P<0.001) on dry matter digestibility (DMD) and organic matter digestibility (OMD), whereas the effect of nutrient density on dietary crude protein digestibility (CPD) only approached significance (P=0.081) (Table 26). No difference (P>0.05) existed for the CPD when the comparison was made between birds fed control diet (T3-100% of standard breed recommendation) and lower nutrient

density (T1and T2, 77 and 85% of standard breed recommendation, respectively), and control diet fed vs. higher nutrient density diets (T4 and T5, 110 and 120% of standard breed recommendation, respectively) fed birds. Control diet fed birds had higher (P<0.01) DMD and OMD almost 12 and 10%, in comparison to birds offered the lower nutrient concentration diets. However, no difference (P>0.05) in DMD and OMD amongst birds existed when the comparison was made between the control diet and higher nutrient density diets (Table 26).

Increasing dietary nutrient concentration led to a linear (P<0.001) improvement in apparent metabolizable energy (AME) and apparent metabolizable energy corrected to nitrogen (AMEn) values of the diets, as AME and AMEn values were reduced for diets T1, T2, T3 and T4 ranged from 34 to 8% lower as compared to T5 diet. Birds fed control diet had higher (P<0.001) dietary AME and AMEn values in comparison to birds offered the lower nutrient concentration diets. However, AME and AMEn values were 9% lower (P<0.01) for the control diet, compared with higher nutrient density fed birds (Table 26). The response of AME intake (AME I) to dietary nutrient concentration was a linear function (P<0.01), where AME I increased with higher dietary nutrient concentration. Birds fed control diet had higher (P<0.001) AME I values in comparison to birds offered the lower nutrient concentration diets, however, no difference (P>0.05) in AME I amongst birds existed when the comparison was made between the control diet and higher nutrient density diets (Table 26).

There was a linear increase (P<0.001) in nitrogen excretion (NEx), nitrogen excretion as part of amino acids (AAN) and nitrogen excretion as uric acid (UAN) as nutrient density increased. On the contrary energy efficiency ratio (EER) positively increased (P<0.001) with lower dietary nutrient concentration, similarly intake of neutral detergent fibre (NDF) increased with a decrease in dietary nutrient density (Table 26). Birds fed diet T1 had significantly higher intake of NDF (P<0.001), almost 134% higher, when compared with the birds fed diet T5 (Table 26). There was a significantly higher (P<0.05) NEx, AAN and UAN was noted when control diet fed birds were compared with lower and higher nutrient density diets fed birds, however, the difference was not significant (P>0.05) for the AAN when comparisons were made between control diet and higher nutrient density diets fed birds (Table 26). There was no difference in EER between the control diet and lower and higher nutrient density diets fed birds. The intake of NDF was significantly higher (P<0.05) when comparisons were made between the control diet and lower nutrient density diets, however, there was a significantly (P<0.001) lower intake of NDF when the control diet was compared with high nutrient density diet (Table 26).

Overall the response of amino acid digestibility (during digestibility measurements after 7th week at 49 days of birds age) i.e. for Ala, Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was best described as positive linear function ($P<0.001$) to dietary nutrient concentration (Table 27). Birds fed the control diet had higher ($P<0.001$) amino acid digestibility in comparison to birds offered the lower nutrient concentration diets. However, amino acid digestibility was either lower or there was a trend of lower ($P<0.05$ to $P=0.09$) values when control birds were compared to birds offered the high nutrient concentration diets, and comparative difference of Val and Met digestibility did not differ ($P>0.05$) between control and lower nutrient density diet fed birds. No difference ($P>0.05$) in digestibility of Arg, Asp, Glu, His, Ile, Leu, Lys, Phe, Ser, Thr, Tyr and Val was noted when control birds were compared to birds offered the high nutrient concentration diets.

Table 20: Effect of dietary nutrient concentration and time on litter moisture (LM), litter ammonia (NH_3, ppm), litter pH (pH), litter temperature (T°) and litter score (LS) parameters.

	Treatments		LM	NH_3	pH	T°	LS
	Diets						
	T1		362.5	6.57	7.74	20.74	2.08
	T2		328.9	6.81	7.85	20.45	1.88
	T3		328.2	8.53	8.21	20.37	1.75
	T4		297.8	8.87	8.15	20.61	1.70
	T5		280.5	9.50	8.12	20.69	1.59
SEM			29.05	0.371	0.069	0.119	0.129
	Time (wks)						
	4-8		225.6	3.21	7.63	21.02	1.43
	8-12		318.0	14.42	8.58	19.83	1.80
	12-16		358.5	9.69	8.13	20.52	2.03
	16-20		376.2	4.90	7.71	20.92	1.94
SEM			9.52	0.268	0.070	0.121	0.044
	Diets	**Time (wks)**					
	T1	4-8	244.0	2.91	7.69	20.98	1.50
	T2	4-8	236.2	3.16	7.49	21.21	1.47
	T3	4-8	232.1	3.73	8.01	20.80	1.44
	T4	4-8	208.7	2.63	7.49	21.11	1.40
	T5	4-8	207.1	3.59	7.47	21.00	1.36
	T1	8-12	348.4	12.50	8.37	20.26	2.07
	T2	8-12	335.1	13.14	8.42	19.61	2.06
	T3	8-12	318.0	14.84	8.64	19.69	1.70
	T4	8-12	302.5	15.07	8.76	19.51	1.69
	T5	8-12	286.0	16.54	8.71	20.06	1.49
	T1	12-16	422.2	7.07	7.53	20.66	2.27
	T2	12-16	355.4	7.07	7.94	20.31	2.15
	T3	12-16	377.8	10.81	8.39	20.19	2.11
	T4	12-16	323.3	10.79	8.40	20.74	1.85
	T5	12-16	313.6	12.71	8.40	20.69	1.76
	T1	16-20	435.5	3.79	7.37	21.06	2.49
	T2	16-20	388.7	3.86	7.55	20.64	1.83
	T3	16-20	384.8	4.71	7.79	20.79	1.76
	T4	16-20	356.7	7.00	7.97	21.09	1.84
	T5	16-20	315.4	5.14	7.88	21.03	1.75
SEM			27.60	0.638	0.152	0.263	0.129
	Probabilities of statistical differences						
Diets			P=0.08	<0.001	<0.001	NS	<0.05
Linear			<0.01	<0.001	NS	NS	<0.001
Quadratic			NS	NS	P=0.06	NS	NS
Contrast 1			NS	<0.001	NS	NS	P=0.07
Contrast 2			NS	NS	NS	NS	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			NS	<0.01	NS	NS	<0.05

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 21: Effect of dietary nutrient concentration and time on leg health parameters i.e. good hock score (GHS), bad hock score (BHS) and total hock score (THS).

Treatments		GHS	BHS	THS
Diets				
T1		0.721	0.279	0.329
T2		0.829	0.171	0.302
T3		0.657	0.343	0.491
T4		0.670	0.330	0.462
T5		0.559	0.441	0.868
SEM		0.0607	0.0607	0.1150
Time (wks)				
4-8		0.456	0.544	0.726
8-12		0.696	0.304	0.501
12-16		0.811	0.189	0.333
16-20		0.559	0.214	0.401
SEM		0.0324	0.0324	0.0493
Diets	**Time (wks)**			
T1	4-8	0.543	0.457	0.543
T2	4-8	0.600	0.400	0.571
T3	4-8	0.500	0.500	0.621
T4	4-8	0.314	0.686	0.800
T5	4-8	0.321	0.679	1.093
T1	8-12	0.757	0.243	0.300
T2	8-12	0.807	0.193	0.371
T3	8-12	0.664	0.336	0.486
T4	8-12	0.771	0.229	0.286
T5	8-12	0.479	0.521	1.064
T1	12-16	0.779	0.221	0.250
T2	12-16	0.936	0.064	0.150
T3	12-16	0.814	0.186	0.314
T4	12-16	0.800	0.200	0.371
T5	12-16	0.729	0.271	0.579
T1	16-20	0.807	0.193	0.221
T2	16-20	0.971	0.029	0.114
T3	16-20	0.650	0.350	0.543
T4	16-20	0.793	0.207	0.393
T5	16-20	0.707	0.293	0.736
SEM		0.0873	0.0873	0.1495
Probabilities of statistical differences				
Diets		P=0.06	P=0.06	<0.05
Linear		<0.05	<0.05	<0.01
Quadratic		Ns	NS	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	NS	NS
Time		<0.001	<0.001	<0.001
Diets x Time		NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 22: Effect of dietary nutrient concentration and time on leg health parameters i.e. good footpad score (GFPS), bad footpad score (BFPS) and total footpad score (TFPS).

Treatments		GFPS	BFPS	TFPS
Diets				
T1		0.876	0.124	0.167
T2		0.879	0.121	0.160
T3		0.867	0.133	0.117
T4		0.857	0.143	0.226
T5		0.905	0.095	0.105
SEM		0.0471	0.0471	0.0805
Time (wks)				
4-8		--	--	--
8-12		0.721	0.279	0.350
12-16		0.970	0.030	0.036
16-20		0.939	0.061	0.079
SEM		0.0308	0.0308	0.0405
Diets	**Time (wks)**			
T1	4-8	--	--	--
T2	4-8	--	--	--
T3	4-8	--	--	--
T4	4-8	--	--	--
T5	4-8	--	--	--
T1	8-12	0.750	0.250	0.350
T2	8-12	0.729	0.271	0.357
T3	8-12	0.664	0.336	0.286
T4	8-12	0.714	0.286	0.479
T5	8-12	0.750	0.250	0.279
T1	12-16	1.000	0.000	0.000
T2	12-16	0.971	0.029	0.029
T3	12-16	0.971	0.029	0.029
T4	12-16	0.943	0.057	0.086
T5	12-16	0.964	0.036	0.036
T1	16-20	0.879	0.121	0.150
T2	16-20	0.936	0.064	0.093
T3	16-20	0.964	0.036	0.036
T4	16-20	0.914	0.086	0.114
T5	16-20	1.000	0.000	0.000
SEM		0.0734	0.0734	0.1090
Probabilities of statistical differences				
Diets		NS	NS	NS
Linear		NS	NS	NS
Quadratic		NS	NS	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	NS	NS
Time		<0.001	<0.001	<0.001
Diets x Time		NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 23: Effect of dietary nutrient concentration and time on leg health parameters i.e. incidences of hock burn (HB) and incidences of footpad dermatitis (FPD), from generalized linear mixed models (GLMM) on logit scale and back transformed on proportion scale (i.e. % of birds with HB>0, FPD>0).

	Treatments	Logit of HB Incidence	Incidence of HB>0	Logit of FPD Incidence	Incidence of FPD>0
	Diets				
	T1	-1.317	21.13	-2.632	6.71
	T2	-2.057	11.33	-2.527	7.40
	T3	-0.799	31.03	-2.856	5.44
	T4	-0.970	27.49	-2.408	8.25
	T5	-0.308	42.37	-2.828	5.58
Min and max SEM		0.5121-0.5510		0.5528-0.5915	
	Time (wks)				
	4-8	0.225	55.59	--	--
	8-12	-1.104	24.89	-1.200	23.15
	12-16	-1.830	13.83	-3.758	2.28
	16-20	-1.651	16.10	-2.993	4.77
Min and max SEM		0.4231-0.4458		0.2772-0.5117	
Probabilities of statistical differences					
Diets		<0.05		NS	
Time		<0.001		<0.001	

There is a statistical significant difference when P<0.05; SEM- standard errors of means (min= Minimum and max= Maximum). The p-values and SEMs are associated with the estimated means on the logit scale of the analysis.

Table 24: Effect of dietary nutrient concentration, time (growth phases) and their interaction on total weight gain ((TWG) kg/b/4 weeks), weight gain ((WG) kg/b/d), feed intake ((FI) kg/b/d), feed conversion efficiency ((FCE) wt gain kg/kg FI) and protein efficiency ratio (PER, wt gain kg/CP intake g).

Treatments			TWG	WG	FI	FCE	PER
Diets							
	T1		4.12	0.147	0.479	0.354	1.84
	T2		4.45	0.159	0.519	0.359	1.96
	T3		4.57	0.163	0.462	0.401	2.03
	T4		4.49	0.160	0.433	0.417	2.13
	T5		4.66	0.166	0.410	0.453	2.12
SEM			0.078	0.0028	0.0146	0.0072	0.105
Time (wks)							
	4-8		3.34	0.119	0.201	0.597	2.49
	8-12		5.00	0.179	0.429	0.419	2.14
	12-16		5.15	0.184	0.600	0.311	1.78
	16-20		4.34	0.155	0.613	0.259	1.66
SEM			0.051	0.0018	0.0069	0.0045	0.033
Diets	**Time (wks)**						
T1	4-8		3.18	0.114	0.208	0.551	2.34
T2	4-8		3.25	0.116	0.211	0.554	2.42
T3	4-8		3.32	0.119	0.201	0.592	2.40
T4	4-8		3.41	0.122	0.194	0.629	2.62
T5	4-8		3.53	0.126	0.192	0.659	2.68
T1	8-12		4.62	0.165	0.446	0.372	1.96
T2	8-12		4.92	0.176	0.456	0.387	2.05
T3	8-12		5.09	0.182	0.425	0.428	2.08
T4	8-12		5.10	0.182	0.420	0.434	2.30
T5	8-12		5.26	0.188	0.396	0.477	2.29
T1	12-16		5.02	0.179	0.632	0.287	1.65
T2	12-16		5.12	0.183	0.663	0.277	1.69
T3	12-16		5.09	0.182	0.583	0.314	1.87
T4	12-16		5.20	0.186	0.582	0.321	1.87
T5	12-16		5.30	0.189	0.541	0.356	1.81
T1	16-20		3.65	0.130	0.632	0.207	1.42
T2	16-20		4.52	0.161	0.747	0.217	1.66
T3	16-20		4.75	0.170	0.640	0.268	1.78
T4	16-20		4.24	0.152	0.534	0.285	1.73
T5	16-20		4.55	0.163	0.512	0.319	1.71
SEM			0.126	0.0045	0.0198	0.0113	0.123
Probabilities of statistical differences							
Diets			<0.001	<0.001	<0.001	<0.001	NS
Linear			<0.001	<0.001	<0.001	<0.001	<0.05
Quadratic			NS	NS	NS	NS	NS
Contrast 1			<0.01	<0.01	<0.05	<0.001	NS
Contrast 2			NS	NS	<0.05	<0.001	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			<0.01	<0.01	<0.001	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 25: Effect of dietary nutrient concentration, time (growth phases) and their interaction on water intake ((WI) kg/b/d), feed intake for water ratio feed (FI W:F) kg/b/d) and water ratio feed ((W:F) kg/kg).

Treatments		WI	FI W:F	W:F
Diets				
	T1	0.843	0.500	1.73
	T2	0.823	0.518	1.69
	T3	0.791	0.479	1.75
	T4	0.738	0.458	1.72
	T5	0.684	0.402	1.81
SEM		0.0381	0.0191	0.050
Time (wks)				
	4-8	0.471	0.219	2.15
	8-12	0.788	0.449	1.76
	12-16	0.855	0.581	1.48
	16-20	0.989	0.635	1.57
SEM		0.0180	0.0101	0.029
Diets	**Time (wks)**			
T1	4-8	0.439	0.227	1.93
T2	4-8	0.459	0.222	2.07
T3	4-8	0.452	0.209	2.15
T4	4-8	0.501	0.224	2.24
T5	4-8	0.506	0.214	2.36
T1	8-12	0.792	0.471	1.69
T2	8-12	0.841	0.478	1.77
T3	8-12	0.858	0.459	1.86
T4	8-12	0.736	0.432	1.71
T5	8-12	0.711	0.402	1.77
T1	12-16	1.004	0.640	1.58
T2	12-16	0.922	0.629	1.48
T3	12-16	0.832	0.581	1.44
T4	12-16	0.767	0.551	1.40
T5	12-16	0.752	0.505	1.50
T1	16-20	1.136	0.660	1.73
T2	16-20	1.070	0.742	1.45
T3	16-20	1.023	0.665	1.53
T4	16-20	0.946	0.624	1.52
T5	16-20	0.768	0.486	1.61
SEM		0.0516	0.0279	0.075
Probabilities of statistical differences				
Diets		<0.05	<0.01	NS
Linear		<0.01	<0.001	NS
Quadratic		NS	P=0.09	NS
Contrast 1		NS	NS	NS
Contrast 2		NS	<0.05	NS
Time		<0.001	<0.001	<0.001
Diets x Time		<0.001	<0.01	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 26: The effect of dietary protein and energy on growth performance, water intake, litter quality and nutrient utilisation parameters.

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Energy efficiency ratio (EER, kg/MJ)	0.054	0.036	0.032	0.034	0.028	0.0056	<0.05	<0.01	NS	P=0.06	NS
N Excreted (g/b/d)	3.810	3.867	4.775	5.184	5.945	0.3170	<0.001	<0.001	NS	<0.05	P=0.05
AAN (g/b/d)	0.935	1.406	1.586	1.599	2.170	0.1586	<0.001	<0.001	NS	<0.05	NS
UAN (g/b/d)	1.521	2.461	3.189	3.585	3.775	0.1934	<0.001	<0.001	<0.05	<0.001	<0.05
NDF I (g/b/d)	18.03	16.29	12.08	9.47	7.17	0.366	<0.001	<0.001	NS	<0.001	<0.001
AME (MJ/kg)	11.53	13.43	15.17	16.04	17.44	0.422	<0.001	<0.001	NS	<0.001	<0.01
AMEn (MJ/kg)	11.40	13.27	14.97	15.84	17.19	0.412	<0.001	<0.001	NS	<0.001	<0.01
AME I (MJ/b/d)	2.07	2.46	2.65	2.71	2.91	0.084	<0.001	<0.001	NS	<0.001	NS
CPD	0.499	0.595	0.597	0.554	0.609	0.0293	P=0.081	P=0.08	NS	NS	NS
DMD	0.587	0.664	0.701	0.709	0.746	0.0241	<0.001	<0.001	NS	<0.05	NS
OMD	0.622	0.690	0.724	0.731	0.766	0.0221	<0.001	<0.001	NS	<0.05	NS

Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) were determined at 49th days of age. However, AME I values represents for growth phase 4-8 weeks were obtained on dry matter basis. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 27: The effect of dietary protein and energy on total tract amino acid digestibility coefficients by turkeys at 8 weeks of age.

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Alanine	0.730	0.782	0.821	0.843	0.871	0.0133	<0.001	<0.001	NS	<0.001	<0.05
Arginine	0.856	0.873	0.903	0.910	0.921	0.0080	<0.001	<0.001	NS	<0.001	NS
Aspartic acid	0.766	0.818	0.842	0.866	0.872	0.0164	<0.001	<0.001	NS	<0.05	NS
Glutamic acid	0.864	0.888	0.895	0.895	0.911	0.0083	<0.01	<0.001	NS	P=0.06	NS
Histidine	0.838	0.867	0.887	0.900	0.894	0.0136	<0.05	<0.01	NS	<0.05	NS
Isoleucine	0.782	0.825	0.856	0.859	0.883	0.0135	<0.001	<0.001	NS	<0.01	NS
Leucine	0.781	0.827	0.858	0.859	0.905	0.0147	<0.001	<0.001	NS	<0.01	NS
Lysine	0.834	0.864	0.896	0.900	0.917	0.0093	<0.001	<0.001	NS	<0.001	NS
Phenylalanine	0.783	0.826	0.852	0.840	0.870	0.0118	<0.001	<0.001	NS	<0.01	NS
Serine	0.819	0.849	0.877	0.879	0.895	0.0102	<0.001	<0.001	NS	<0.01	NS
Threonine	0.805	0.845	0.871	0.874	0.892	0.0099	<0.001	<0.001	NS	<0.001	NS
Tyrosine	0.816	0.857	0.881	0.889	0.905	0.0104	<0.001	<0.001	NS	<0.01	NS
Valine	0.731	0.787	0.822	0.831	0.868	0.0163	<0.001	<0.001	NS	<0.01	NS

Amino acids digestibilities were determined at 49th days of age. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 28: Correlation matrix for bird performance, litter quality, dietary nutrient digestibility, and leg health in response changes in nutrient density.

	FI	WG	FCE	WI	W:F	LS	LM	NH_3	CPD	DMD	HBS
WG	-0.490										
FCE	-0.918	0.787									
WI	0.890	-0.757	-0.980								
W:F	-0.808	0.486	0.796	-0.733							
LS	0.732	-0.941	-0.933	0.920	-0.595						
LM	0.737	-0.846	-0.915	0.959	-0.549	0.955					
NH_3	-0.882	0.817	0.972	-0.935	0.671	-0.953	-0.900				
CPD	-0.176	0.929	0.545	-0.522	0.344	-0.760	-0.657	0.552			
DMD	-0.666	0.968	0.899	-0.885	0.555	-0.996	-0.940	0.924	0.814		
HBS	-0.831	0.709	0.922	-0.906	0.930	-0.810	-0.806	0.813	0.561	0.781	
FPS	0.128	-0.415	-0.283	0.185	-0.663	0.252	0.106	-0.167	-0.560	-0.280	-0.557

d.f. = 33 Correlation coefficients greater than 0.349 and 0.449 are statistically significant at 5% ($P<0.05$) and 1% level ($P<0.001$), respectively.
Key:FI (feed intake), WG (weight gain), FCE (feed conversion efficiency), WI (water intake), W:F (water to feed ratio), LS (litter score), LM (litter moisture content), NH_3 (ammonia in litter), CPD (crude protein digestibility), DMD (dry matter digestibility), HBS (hock burn scores) and FPS (footpad dermatitis scores).

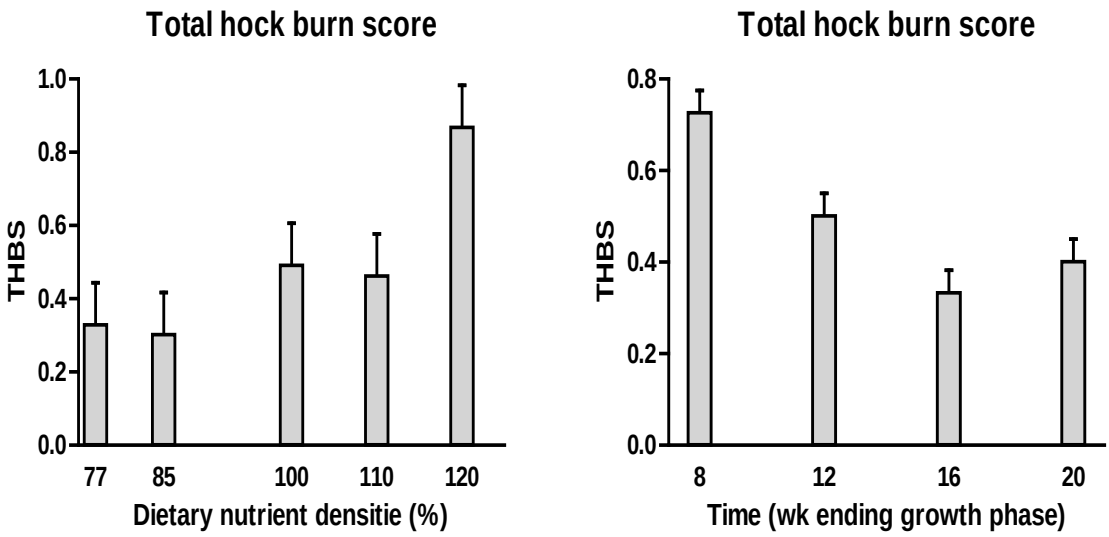


Figure 17: The effect of nutrient density and time (growth phases) on the total hock score (THS) in 20 week old turkeys (error bars represents pooled SEM).

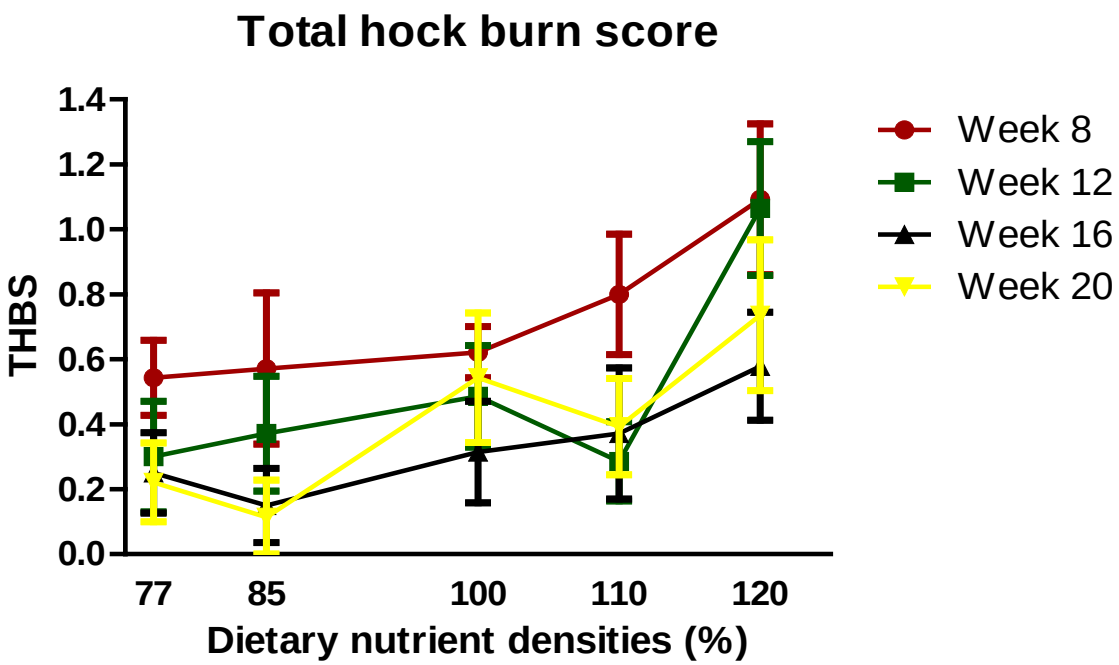


Figure 18: The effect of dietary nutrient density and growth phases on the trend of total hock score (THS) in 20 week old turkeys (SEM bars correspond to each data point).

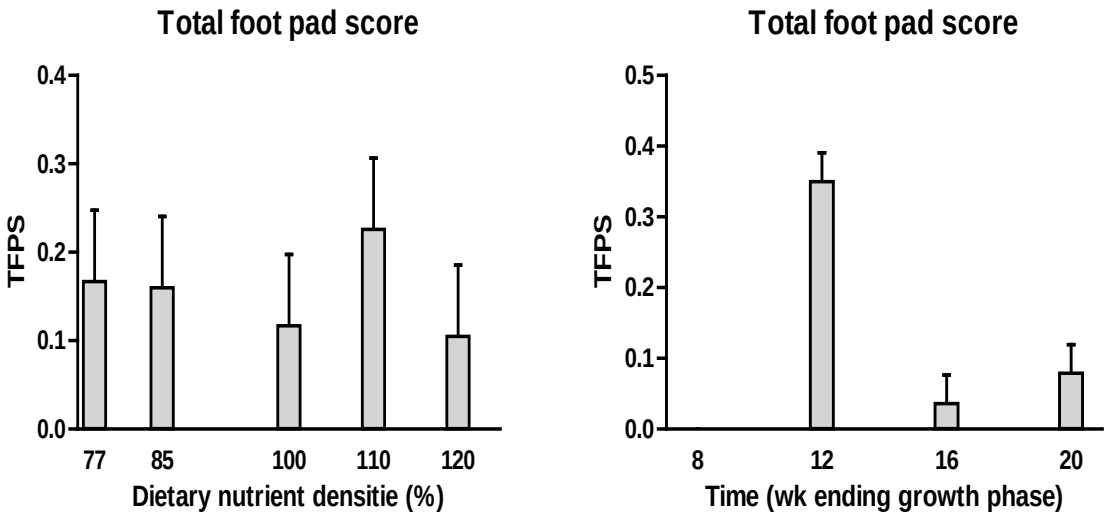


Figure 19: The effect of nutrient density and time (growth phases) on the total foot pad score (TFPS) in 20 week old turkeys (error bars represents pooled SEM).

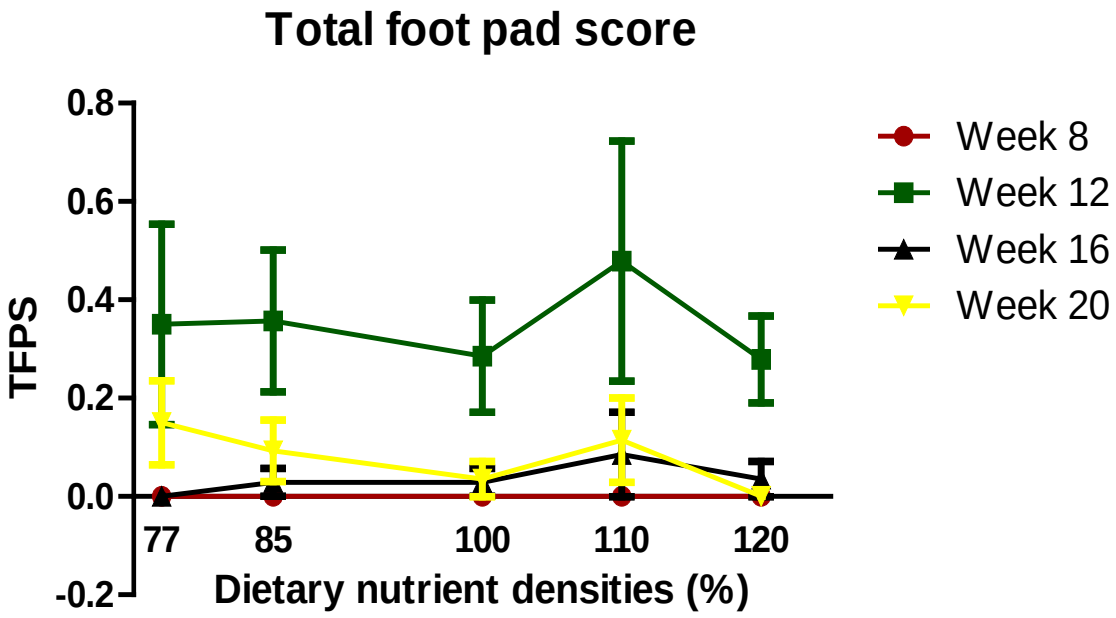


Figure 20: The effect of dietary nutrient density and growth phases on the trend of total foot pad score (TFPS) in 20 week old turkeys (SEM bars correspond to each data point).

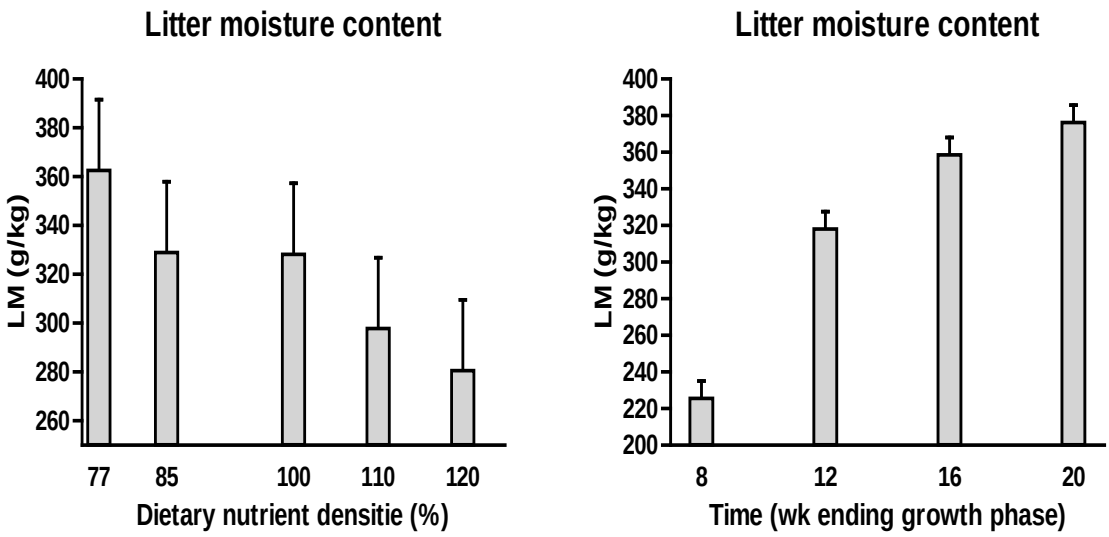


Figure 21: The effect of nutrient density and time (growth phases) on the litter moisture content (LM) in 20 week old turkeys (error bars represents pooled SEM).

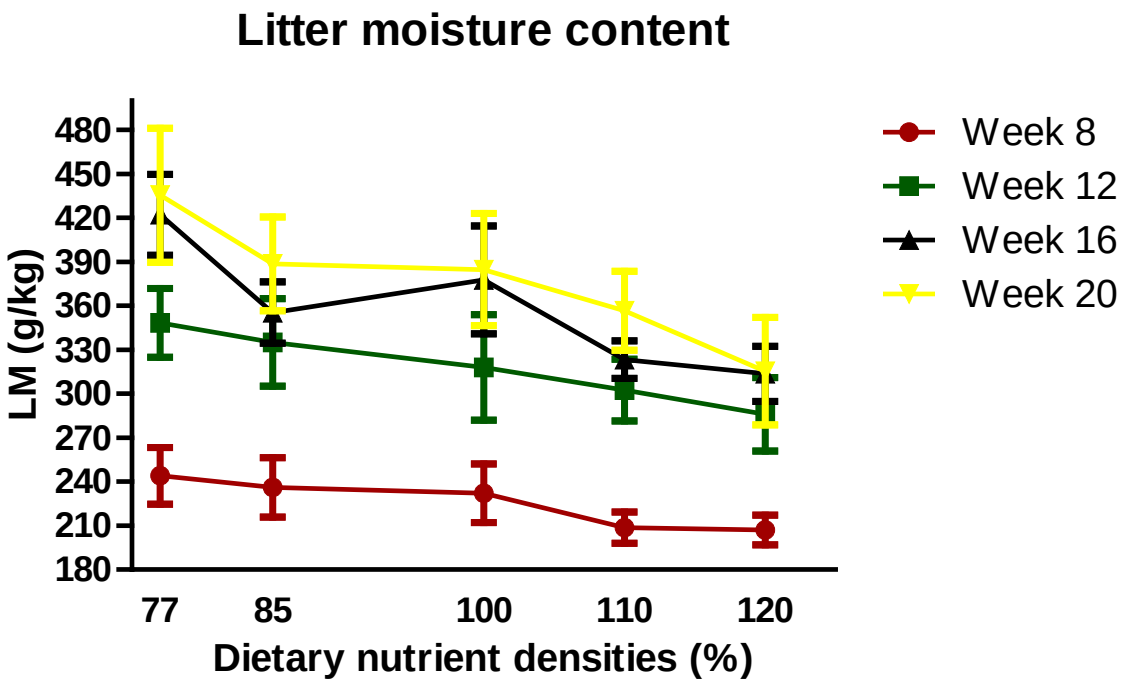


Figure 22: The effect of dietary nutrient density and growth phases on the trend of litter moisture content (LM) in 20 week old turkeys (SEM bars correspond to each data point).

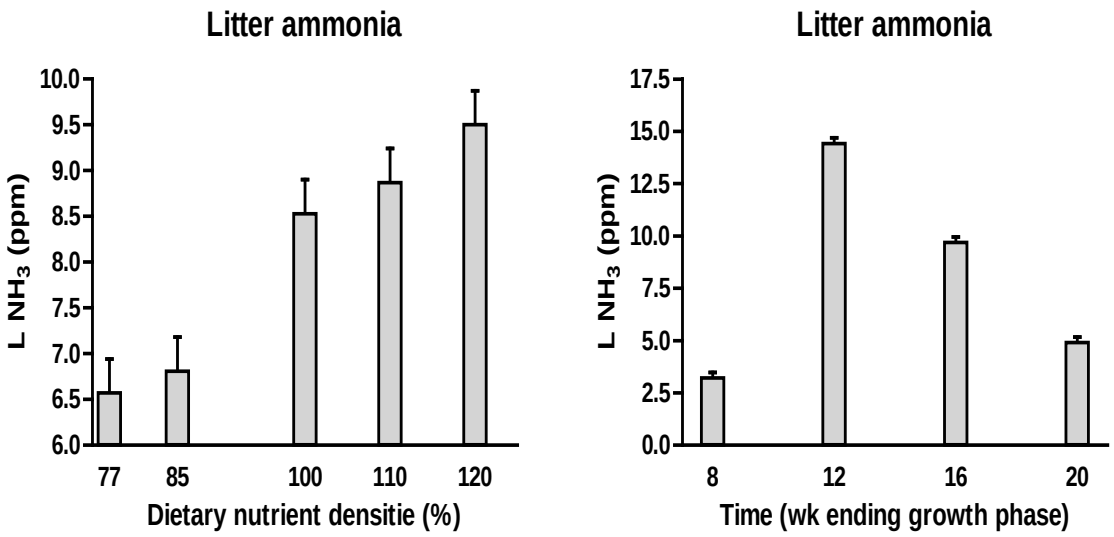


Figure 23: The effect of nutrient density and time (growth phases) on litter ammonia (L NH_3) in 20 week old turkeys (error bars represents pooled SEM).

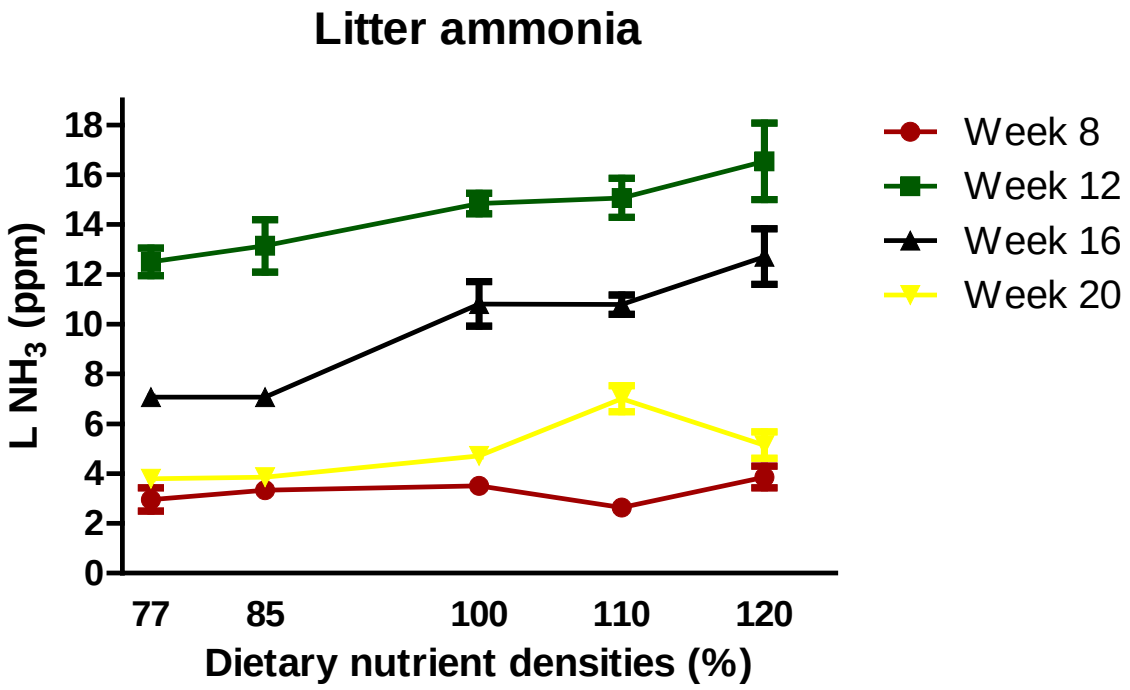


Figure 24: The effect of dietary nutrient density and growth phases on the trend of litter ammonia (L NH_3) in 20 week old turkeys (SEM bars correspond to each data point).

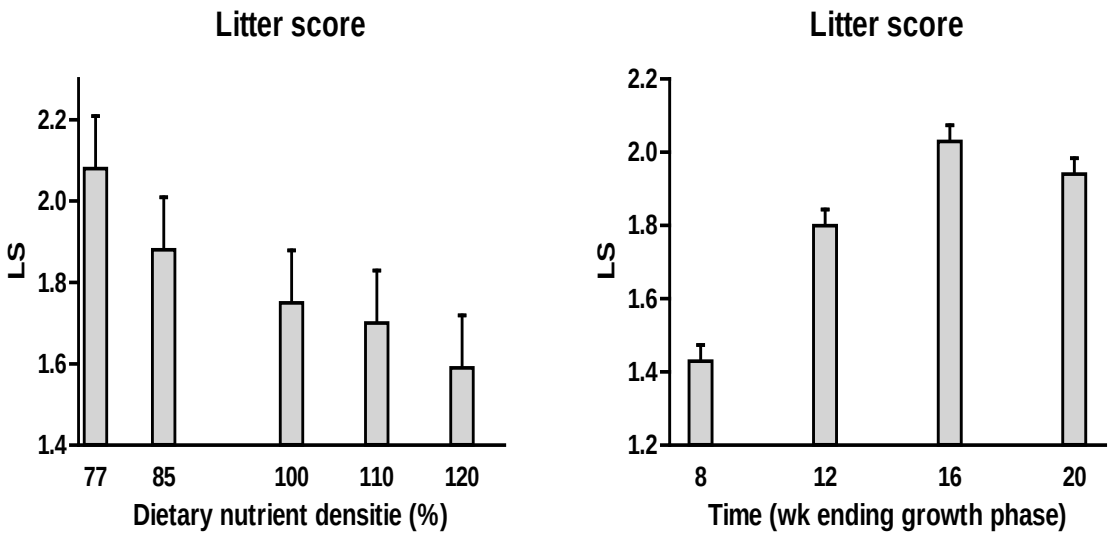


Figure 25: The effect of nutrient density and time (growth phases) on the litter score (LS) in 20 week old turkeys (error bars represents pooled SEM).

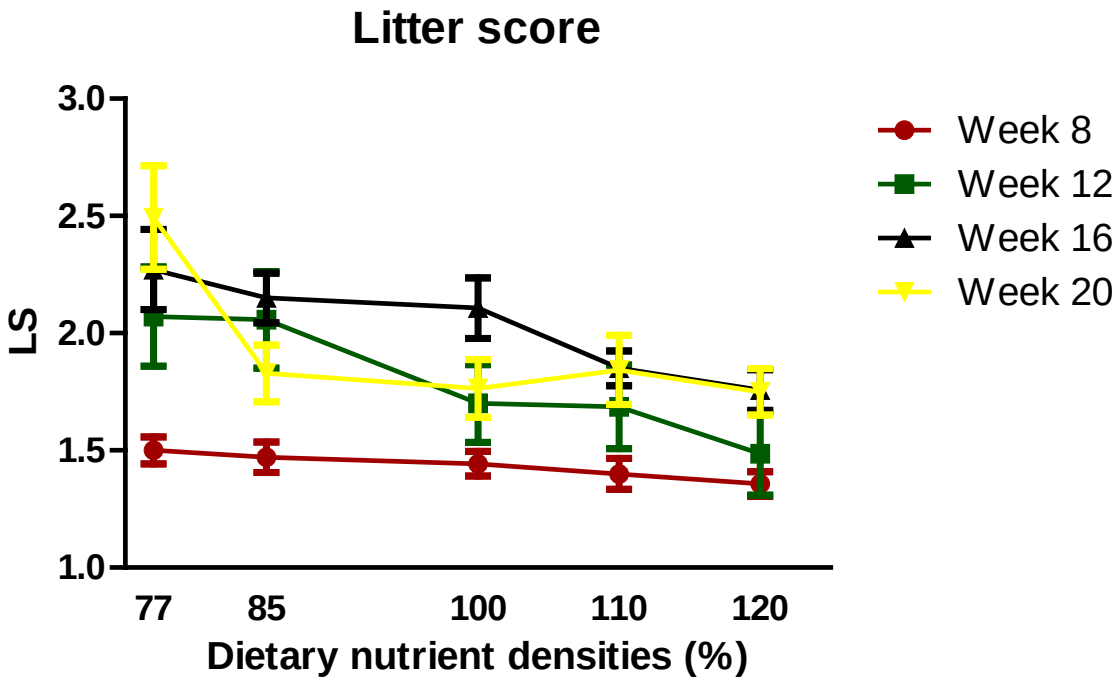


Figure 26: The effect of dietary nutrient density and growth phases on the trend of litter score (LS) in 20 week old turkeys (SEM bars correspond to each data point).

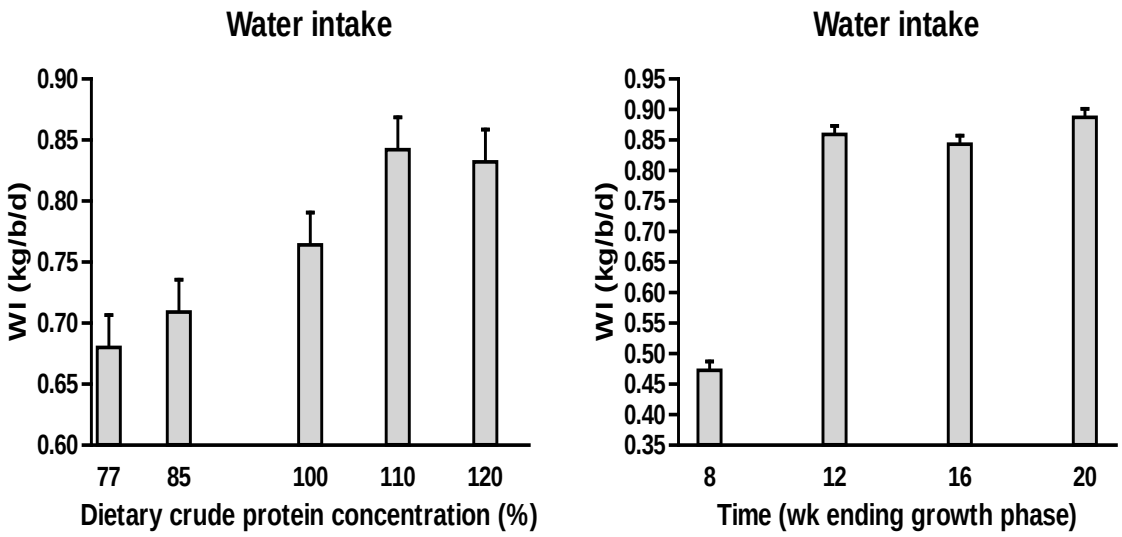


Figure 27: The effect of nutrient density and time (growth phases) on the water intake (WI) in 20 week old turkeys (error bars represents pooled SEM).

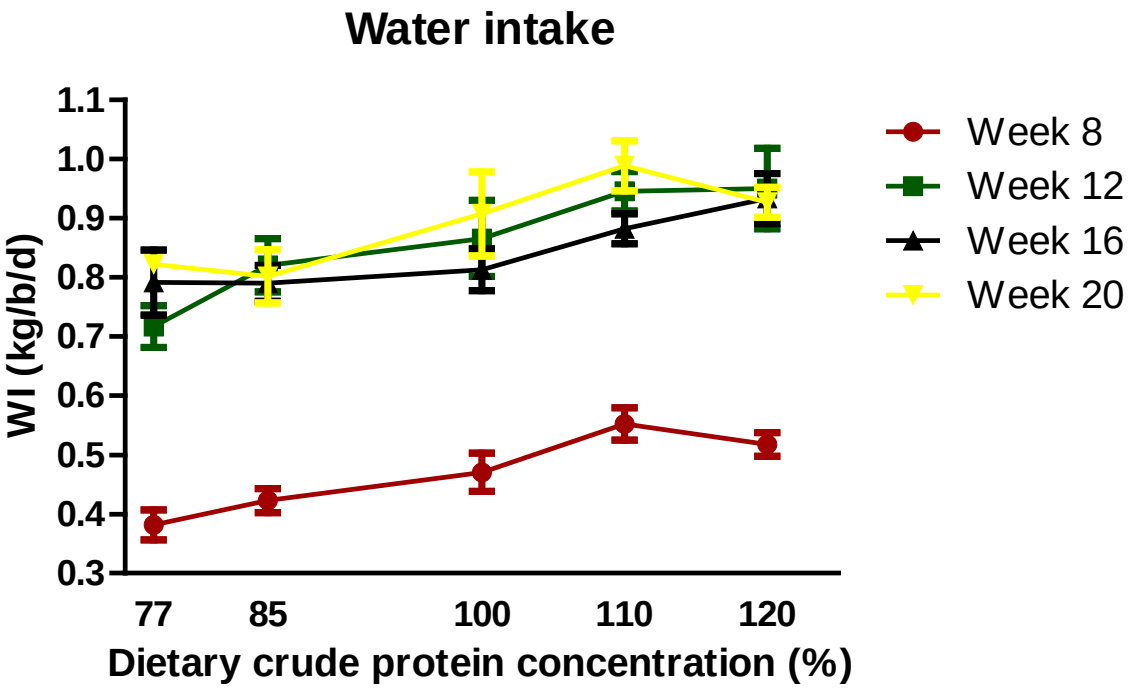


Figure 28: The effect of dietary nutrient density and growth phases on the trend of water intake (WI) in 20 week old turkeys (SEM bars correspond to each data point).

3.4 **Discussion**

The analysed dietary concentration of crude protein (CP) were slightly lower and the values for K, Ca and Na concentration were higher than the calculated values, which was probably due to differences between the composition of the actual ingredients that were used in the present study and the NRC (1994) values for the same ingredients. The relatively higher final body weight of the birds, when compared to breed standards, may be explained by the 'small pen' effect, e.g. a reduction in competition for, and closer proximity to, drinkers and feeders.

3.4.1 Water intake measurements

At moderate temperatures feed intake, or more specifically dry matter intake, is the main determinant of the daily water requirement of poultry (Pond *et al*., 1995). However water intake and the ratio of water to food intake are increased by high dietary mineral and protein concentrations (Fuller *et al*., 2004). In order to maintain water balance, water intake must exactly counterbalance the water lost from the body as well as water stored in new growth therefore any over consumption from the requirement can lead to higher than normal water excretion. Since the dietary concentration of nutrients other than CP and AME were kept similar in all dietary treatments, however, NDF content changed significantly due to feed formulation constraints in the lower nutrient density diets, therefore, higher feed intake resulted in a higher mineral and NDF intake, which are known to increase water intake and excretion in poultry (Van der Klis *et al.*, 1995). Therefore as expected higher feed intake (FI) in the present study in birds fed on lower nutrient density diets resulted in higher water intake (WI) which then resulted in poor litter quality.

Feed intake and feed composition can affect metabolism and utilisation of individual amino acids which then can affect normal gut functioning and can impair absorption of other nutrients. Certain dietary factors such as fibre, lignins, tannins and lectins can influence threonine availability to the animal. It has been shown in the literature that threonine deficiency caused by either inadequate dietary supply or due to factors mentioned above can result in increased excretion of mucins and abrasion leading to severe diarrhoea in pigs (Law *et al.*, 2007). Higher level of dietary NDF in poor nutrient density fed birds of present study could have resulted in poor absorption of nutrients across GIT, hence resulted in higher retention within digesta. In the present study lower amino acid digestibility in diets where nutrient density was lowest therefore, indicates that the dietary NDF content in diets formulated with lower nutrient density might have been the cause of lower amino acid digestibility and imbalance. An amino acid imbalance is

highly likely to make things worse when compared with a well balanced amino acid profile (D'Mello, 1993; D'Mello, 1994; Moran & Stilborn, 1996). Symptoms of imbalance or deficiency of linoleic acid in the domestic fowl include retarded growth, increased water consumption (Stevens, 2004). Higher NDF intake in birds fed with lower nutrient density diets in the present study created a severe imbalance of amino acids causing a reduction in protein utilisation and a lower FCE. Fibre itself is responsible for decreased protein digestibility in pigs, with water retention capacity being shown to increase ileal protein losses (Larsen *et al.*, 1993). It has been reported by Fairclough *et al*. (1980) that free amino acids exert more osmotic pressure than peptides, and free amino acids may in some cases be utilized even less efficiently than protein-bound amino acids (Boisen, 2003). Therefore, this situation could lead to excretion of water more than normal through excreta as reported in the present study. Diarrhoea can affect the availability of other amino acids (e.g. methionine) required for gut function and metabolism. For example, threonine is regarded as crucial for normal gut structure and function so its requirement is quite high. Pigs can use almost 60% of their threonine intake for gut development and functioning (Stoll *et al.*, 1998). Since threonine is required for gastrointestinal secretions (mucin) that protect mucosa from digestive proteases, dehydration, microbial and parasitic invasion and therefore, believed to play an important role in development and normal functioning of the gut (Bertolo *et al.*, 1998; Stoll *et al.*, 1998). Likewise any imbalance or improper supply of other amino acids such as leucine can affect gut functioning and structure. Adequate arginine intake is crucial for normal metabolic function in pigs and any deficiency can result in increased plasma ammonia concentration leading to metabolic disturbance (hyperammonemia) (Urschel *et al.*, 2007). These problems can be addressed by dietary supplementation of arginine (Zhan *et al.*, 2008). As it is required for the synthesis of protein, urea, nitric oxide and other metabolites and any inadequate supply for one or the other reasons can change the priority of its usage. This can result in higher concentration of ammonia in the plasma which is toxic and required more water for excretion as explained in Section 1.10.1.3.1. It is also documented in the literature that higher feed and mineral intake can depress DMD (Koreleski *et al.*, 2010) and amino acid absorption.

Further to amino acid imbalance and digestibility association with litter quality problems, undigested starch and protein favour proliferation of coliform bacteria in pigs (Jeaurond *et al.*, 2008). However, fibre can reverse the ratio of coliform bacteria to other beneficial bacteria (lactobacilli) and can reduce ammonia contents in GIT (Bikker *et al.*, 2006). But it is worth noting that source of fibre can produce different affects as fibre from wheat bran provides intermediate results.

Goldstein & Skadhauge (2000) highlighted that lower protein fed birds when had limited dietary energy available can have relatively higher quantity of nitrogen excreted in forms other than uric acid it is just to conserve energy. These forms e.g. urea and ammonia are osmotically active and require alot of water to be excreted. The lower dietary energy and its relationship with higher amino acids being oxidsed to be used as energy source were explained (Church, 1991; Pfeiffer, 1995; Musharaf & Latshaw, 1999) highlighting the fact that it is not the absolute dietary CP but the ratio between ME and CP is perhaps more important when a control on litter moisture and nitrogen is to be ensured. Caution is therefore necessary in reaching any conclusions when evaluating studies referring to relationship of dietary CP with litter moisture contents.

3.4.2 Litter quality associated parameters

An increase in nutrient density resulted in a reduction in the litter moisture (LM) content and this relationship suggested that the optimum dietary nutrient density for reduced LM does not match with the determined optimal density for bird growth. Therefore, the higher LM content reported in this study could have been the reflection of higher nutrient retention in digesta possibly due to poor DMD, OMD, amino acid digestibilities and presence of higher NDF content, when birds were fed lowest level of dietary energy and protein concentrations. However, present findings differ to some extent from findings reported by Khajali & Moghaddam, (2006) that there was no effect of lower dietary crude protein concentration on litter moisture content. However, they are in agreement with present findings of reduction in nitrogen excretion when birds were fed lower dietary protein concentration.

In terms of nitrogen excretion by the bird and a reduction in the litter NH_3 concentration these results are in line with previous findings of different studies which reported that a reduction in dietary protein content can help control nitrogen excretion and NH_3 emission from poultry litter (Jacob *et al.*, 1994; Moran & Stilborn, 1996; Ferguson *et al.*, 1998; Hussein *et al.*, 2001; Bregendahl *et al.*, 2002; Rezaei *et al.*, 2004; Si *et al.*, 2004). Uric acid is the end product of protein degradation in avian species and is a direct measure of protein catabolism in birds. Some researchers reported a decrease in uric acid concentration in the blood when lower protein diets were fed to broilers (Rosebrough *et al.*, 1996; Collin *et al.*, 2003). Different researches (Cheng *et al.*, 1997; Aletor *et al.*, 2000; Swennen *et al.*, 2004; Swennen *et al.*, 2005; Swennen *et al*. 2006) have reported that birds have mechanism to reduce amino acid oxidation as a sparing mechanism which therefore, is the reason of lower plasma uric acid level. Therefore, probable reason of this lower litter NH_3 content was due to the lower uric acid excretion by the birds fed on lower nutrient density diets.

3.4.3 Leg health parameters

Increasing litter score (reflecting deterioration in litter quality) had a positive correlation with WI however, the negative correlation of WI with hock burn scores (HBS) may appear contrary to previous findings (Mayne *et al.*, 2007), because it might be expected that high water intake would result in poor litter quality or high LM with a resulting increase in contact dermatitis. The reduced litter moisture and lower litter scores were achieved with an increase in nutrient density which is in agreement with the findings of Kenny *et al*. (2010). However this improvement in litter quality did not correspond with the incidence of HB or FPD. The higher incidences of HB were associated with birsd fed the higher nutrient density diet, in agreement with the findings of Bilgili *et al*. (2006). The positive correlation of HB with litter NH_3 indicates that perhaps litter chemical properties are important contributor in skin damage and litter moisture may only aggravate the damage by making skin more prone to these damages. Therefore, present findings suggested that it may be the litter NH_3 and pH which has a much greater effect on incidence of hock burn than litter moisture content alone. Therefore, in terms of HBS it was notable that increases in litter moisture were not associated with increased HBS. It is likely that the cause of the higher HBS in groups fed higher nutrient density diets was primarily litter NH_3. Unlike Ekstrand *et al*. (1997) and (1998) litter moisture was the main cause of footpad dermatitis (FPD). However, Dawkins *et al*. (2004) reported that a combination of litter moisture and ammonia was associated with poor health and correlated with ‘dirty foot pads’. Berg (2004) also noted that HB lesions are commonly caused by a combination of moisture, high ammonia content, and other unspecified chemical factors in the litter. There is another possible reason for higher incidences of HB in birds fed the higher nutrient density diets. These birds may spend less time standing for feed and therefore, spend more time sitting on the litter. Haslam *et al*. (2007) reported that factors which increase bird weight or which are related to reduced litter quality, tend to increase hock burn.

Although litter moisture increased with age in this study there was a reduction in the HBS as well as FPDS which highlights that it is not litter moisture alone that can cause skin damage. These findings agree with the findings of Bilgili *et al*. (2006) who reported that the proportion of birds with footpad dermatitis tended to increase until 49 days of age after which they started to decline. So it is possible that older birds may become less susceptible to litter moisture damage (Mayne *et al*., 2007).

The findings in this study contrast with those of Mayne *et al*. (2007), who reported that litter moisture was the cause of FPD in turkeys. Increased litter moisture not associated with more incidences of FPD although these findings may be consistent with those of

Dawkins *et al*. (2004) who concluded that both litter moisture and NH_3 are required to predispose birds to FPD rather than litter moisture alone.

3.4.4 Growth performance, dietary nutrient intake and utilisation

It is well documented that dietary composition and the ratios between macronutrients have a major impact on performance and body composition of chickens (Macleod, 1990; Macleod, 1992; Nieto *et al.*, 1997; Collin *et al.*, 2003). In the present study birds fed on lower nutrient density had lower crude protein digestibility (CPD) as well as lower feed conversion efficiency (FCE) and protein efficiency ratio (PER) which are consistent with previous reports. For example, some studies have reported a negative effect on feed conversion ratio of lower crude protein concentration even when supplemented with synthetic amino acids (Moran & Stilborn, 1996; Ferguson *et al.*, 1998; Neto *et al.*, 2000). Layer birds eat to meet their energy requirement, so physical capacity and energy content can affect both feed intake (Morris, 1968; Golian & Maurice, 1992; Leeson *et al.*, 1993). Study of Huang *et al*. (2009), the present findings suggest that meat producing birds also try to compensate for any energy deficiency by increasing their feed intake when fed a lower nutrient density diet however, in this study, they were not able to match the similar weight gain as recored in birds fed with higher nutrient density diets. The lower weight gain and poor feed conversion efficiency in the present study in birds fed on lower nutrient density was consistent with Hidalgo *et al*. (2004) who reported the same when broilers were fed diets with suboptimal levels of energy and crude protein while maintaining ME:CP. Farrell *et al*. (1973) and Farrell (1974) suggested that there is an optimum energy concentration in the diet beyond which the performance of birds does not appear to improve and that in some cases, it may actually deteriorate. The present findings agree with this conclusion only during the last growth phase (16-20 weeks) where maximum weight gain was recorded when birds fed with diet contain 100% nutrient density compared to either of the lower or higher nutrient density diet fed birds.

Others reported a reduced growth performance with a reduction of as little as 30g/kg dietary crude protein concentration even when the diet was supplemented with synthetic amino acids (Fancher & Jensen, 1989a; Fancher & Jensen, 1989b; Fancher & Jensen, 1989c; Pinchasov *et al.*, 1990; Colnago *et al.*, 1991; Kerr & Kidd, 1999; Aletor *et al.*, 2000; Waldroup, 2000; Bregendahl *et al.*, 2002). Whereas Aletor *et al*. (2000) reported improved protein efficiency ratio with lower dietary crude protein concentration because dietary protein is preferentially used for protein deposition. However, other studies also indicated the importance of dietary energy concentration along with CP as they reported poor protein deposition in the carcass in case the energy availability becomes limiting (Macleod, 1990; Musharaf & Latshaw, 1999).

Overall decrease in FCE, PER and an increase in feed intake (FI) with age in the present findings can be best explained by the fact that birds are able to retain more protein at younger age and with the age this ability decrease and they retain more fat. Fat contains more energy than protein and gaining body fat require more feed intake to be converted to less body growth compared to protein.

The experimental diets were formulated to contain graded levels of dietary energy and protein concentrations, because, it was hypothesised, would affect feed and water intake and hence litter quality and would allow test of their response to different dietary concentrations. However, the overall changes in growth performance parameters were expected, i.e. most of the dietary energy and protein concentrations were beyond those used in commercial practice, therefore, they are not further discussed in this chapter.

The higher energy efficiency ratio (EER) in birds fed lower nutrient density diets seems to be at variance from the FCE and PER results. However, this can be explained by the uric acid excretion values of birds fed lower nutrient density diets being lower than for those birds fed on higher nutrient density diets. As explained in Section 1.10.1.3.1, uric acid formation and excretion is a process that requires significant energy. Therefore, birds fed on higher nutrient density diets use energy on uric acid excretion, hence had lower EER values. The present findings agree with the findings of Skinner *et al*. (1992) who reported that an increase in dietary nutrient density resulted in depressed energy efficiency.

Poor nutrient utilisation i.e. CPD, dry matter (DM), organic matter (OM) and amino acid digestibilities in birds fed lower nutrient density diets in the present study could be explained by the presence of higher concentration of neutral detergent fibre (NDF) in the diets formulated to present lower nutrient concentrations. The proportion of cellulose and lignin in the crude fibre fraction also determines the digestibility of crude fibre or its solubility in the intestine. AWT (2005) report by-products of cereal processing such as wheat bran to be particularly high in fibre while soybean meal (especially high protein grades) bring little fibre into the formulation (e.g. pentosans i.e. arbinose and xylose etc. wheat bran 250 g vs. 35 g/kg DM in soybean meal). Since fibre has no direct nutritive benefit in poultry nutrition the high cellulose and lignin concentrations as result of formulation constraint to add wheat bran could have resulted in reduced nutrient digestibility.

3.5 **Conclusion**

The present experiment has shown that an increase in the concentration of dietary crude protein (CP) and apparent metabolisable energy (AME) can reduce water intake (WI), decreasing moisture content in the litter and thereby reduce the litter score (indicating improved overall litter quality). However, the incidence of hock burn increased with the high nutrient density diets, suggesting that factors other than the litter moisture alone may contribute the occurrence of leg health (defined in this study as FPD and HB) problems in turkey production.

The incidence of hock burn (HB) was associated with litter NH_3. Since CP intake was related to litter NH_3 concentration, then modifying the CP intake by altering the calorie to CP ratio may be one way of controlling HB by dietary manipulation.

It is perhaps important to report that good litter score (based on physical appearance) was not related to litter NH_3 and pH therefore litter score per se is of limited or no value in terms of lowering HB incidences in turkey production.

Chapter 4

Effect of higher energy to protein ratio on on litter moisture content and FPD in growing turkeys

4 Aim

The objectives of this experiment were to examine the effects of different protein concentrations (with ideal amino acid ratio) with a constant metabolizable energy content (providing varying ME:CP) on:

- water intake and excretion of male turkeys
- litter quality
- FPD
- growth performance, dietary nutrient intake and utilisation

4.1 Background

Maintaining litter quality is essential if the prevalence of conditions such as pododermatitis (FPD) and hock burn (HB) are to be minimised. One of the main factors that influences the quality of the litter is its moisture content, levels of <25% being consistent with good quality litter (Collett, 2009). The causes of wet litter are multi-factorial, reflecting the interaction between nutrition, management and intestinal health (Lister, 2009).

A strong correlation between dietary crude protein (CP) and litter moisture levels and nitrogen excretion was indicated by studies e.g. (Marks & Pesti, 1984; Pfeiffer *et al.*, 1995; Alleman & Leclercq, 1997; Ferguson *et al.*, 1998; Clark *et al.*, 2002; Furlan *et al.*, 2004; Rezaei *et al.*, 2004; Ziaei *et al.*, 2007). Dietary CP concentration can affect the welfare, economic return and meat quality of poultry and this makes it difficult to adjust its concentration in the diet of turkeys where the requirement is very high as compared to other poultry birds (Eits *et al.*, 2004; Kidd *et al.*, 2004). Formulating turkey diets on an ideal protein basis is believed to be the best solution to meet the animals requirement for protein accretion and maintenance while avoiding any deficiency and excess which can increase feed cost and nitrogen excretion (Moran & Stilborn, 1996; Parsons, 1996; Emmert & Baker, 1997; Heger *et al.*, 1998; Firman & Boling, 1998; Baker *et al.*, 2003; Firman, 2004; Lemme *et al.*, 2004; Waldroup *et al.*, 2005a). Dietary imbalance of amino acids can also affect performance parameters such as feed intake and weight gain (Sklan & Plavnik, 2002; Namroud *et al.*, 2008).

Since NH_3, high litter moisture content and quality are correlated with dirty footpads, FPD and hock lesions in poultry (Ekstrand *et al.*, 1997; Dawkins *et al.*, 2004; Haslam *et al.*, 2006; Mayne *et al.*, 2007). This study aims to establish how these dietary modifications can affect litter characteristics like moisture, pH and NH_3 content and the correlation of these characteristics with the FPD and Hock burns in turkeys.

Most studies on the influence of nutrition on litter quality have been conducted with broiler chickens and there is relatively little information on the effect of dietary protein levels on litter quality in turkey production. The experiments described in Chapters 2 and 3 show that absolute dietary nutrient concentration can affect litter moisture content and NH_3 contents. Since the second experiment (Chapter 3) suggested that there are interactions between aspects of litter quality such as NH_3 and moisture with leg health. Therefore, this third experiment was designed to investigate and establish whether the ratio between dietary energy and protein is important as well. In this experiment, it is hypothesized that the ratio of energy to protein, as well as the absolute levels of these in the diet, is important for reducing litter moisture and NH_3 content and therefore, incidences of leg health problem.

4.2 **Material and methods**

All the methodology was same as mentioned earlier in Sections from 3.2.1 to 3.2.13.1 except Section 3.2.2 which is as follows:

4.2.1 Feed preparation

In the pre-study period, from 0 to 4 weeks of age, the birds were fed a standard crumb starter turkey feed (Table 7). The starter diet consisted of major feed ingredients such as wheat, soybean meal, and fish meal containing crude protein (CP) 263 g/kg and metabolisable energy (ME) 12.15 MJ/kg.

Five experimental diets in total were used for each growth phase (4 weeks each and starting at 4 weeks of turkey's age till 20 weeks) in the study. The wheat-soybean based diets in pelleted form were prepared according to the formulation for BUT 8 (Aviagen Turkeys Ltd., UK) given below (Table 30 toTable 33). Diet T3 served as control with 100% of CP and ME according to BUT 8 requirement for each growth phase. While diets T5, T4, T2 and T1 contained 120, 110, 85 and 77% concentration of CP respectively as compared to control diet T3. All the diets were isocaloric (MJ/kg) according to the respective growth phase nutrient and recommendation of BUT 8. Digestible amino acid profile was similar during a growth phase of 4 weeks for all the diets according to BUT 8 recommendations

with some missing values obtained from Firman & Boling (1998) and upgraded according to commercial values (Table 8). Amino acids like lysine, methionine and threonine were included where deficient to meet the requirement. All the rest of the nutrient remains the same according to BUT 8 commercial nutrient requirements for that particular growth phase. Each experimental diet for the respective growth phase was fed randomly to selected seven replicates for the period from 4 to 20 weeks. All diets were offered as pelleted. The diets used for experiment were analysed for their dry matter (DM), CP, minerals, crude fat (EE), neutral detergent fibre (NDF), ash, ME and amino acid content.

The methodology for DM, Ash, nitrogen and gross energy determinations were described in Sections 2.2.3 and 2.2.4. The fat content was determined with AOAC 920.39 method using a Soxtec 1043 extraction unit (Foss Ltd, Wigan, UK). The dietary neutral detergent fibre (NDF) fraction was determined according to procedure described by Holst (1973).

The methodology for feed conversion efficiency (FCE) and for protein efficiency ratio (PER) was described in Section 2.2.8 however, the units for weight gain and CP intake was kg instead of g. Whereas energy efficiency ratio (EER) was calculated as weight gain (kg/d) / AME intake (MJ/d). The methodology for determination of nutrient digestibility coefficients calculations were used as described in Section 2.2.9 but for, amino acid digestibility coefficients the equations were modified for each amino acid described in Section 2.2.9.

The methodology for water intake, feed intake and body weight measurements was described in Chapter 3 Section 3.2.4, 3.2.5 and 3.2.6, respectively. Whereas methods for excreta collection, footpad scoring, hock burn scoring, litter scoring, litter pH and NH_3, litter analysis, amino acid and mineral determinations from feed and excreta was described in Chapter 3 Section 3.2.7, 3.2.8, 3.2.9, 3.2.10, 3.2.11, 3.2.12, 3.2.13.1 and 3.2.13.2, respectively.

Table 29: Ingredient composition (g/kg) of the starter diet fed to the turkeys during the pre-study period from 0 to 4 weeks of age.

Ingredients	g/ kg
Fish meal - (72% - CP)	30
Soybean meal - (48% - CP)	275
Wheat	575
Corn gluten - (60% - CP)	20
Casein	30
Soy oil	17.4
Lysine HCl	1.9
DL Methionine	2.8
L-Threonine	3.9
Salt	2.2
Limestone	7
Dicalcium phosphate	21.5
Vit/min Premix[1]	2.8
Coccidiostat	0.5
Pellet binder	10
Calculated nutrient analysis	
Metabolisable energy (ME), MJ/kg[2]	12.15
Crude protein (CP) (g/kg)	263.1
Crude fibre (g/kg)	29
Ca (g/kg)	10
Available Phosphorus (g/kg)	5
Na (g/kg)	1.5
Cl (g/kg)	2.3
K (g/kg)	8.2
Indispensable amino acids	
Arginine (g/kg)[3]	12.2
Cystine(g/kg)[3]	4.2
Isoleucine (g/kg)[3]	9.6
Lysine(g/kg)[3]	13.1
Methionine(g/kg)[3]	5.1
Phenylalanine(g/kg)[3]	10.5
Threonine(g/kg)[3]	8.1
Tryptophan (g/kg)[3]	3.1
Valine(g/kg)[3]	10.4
Dispensable	
Tyrosine (g/kg)[3]	9.4

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 30: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 4-8 weeks of age.

Ingredients	Crude protein concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%- CP)	30	31.9	35.4	37.7	40
Soybean Meal - (48%- CP)	140	188.5	277.9	336.7	395.4
Wheat, White	737.9	679.5	571.8	501	430.3
Corn gluten meal - (60% - CP)	0	3.8	10.8	15.4	20
Casein	30	30	30	30	30
Soybean OiL	10	14.1	21.6	26.6	31.5
L-Lysine HCl	2	1.9	1.7	1.6	1.5
DL-Methionine	2	2.3	3	3.4	3.8
L-Threonine	2.1	2.8	4	4.8	5.6
Common Salt	2.2	2.2	2.2	2.2	2.2
Limestone	6.8	6.6	6.1	5.8	5.5
Dicalcium phosphate	23.3	22.8	21.8	21.1	20.5
Vit/min Premix[1]	3.2	3.2	3.2	3.2	3.2
Coccidiostat	0.5	0.5	0.5	0.5	0.5
Pellet binder	10	10	10	10	10
Calculated nutrient analysis					
ME (MJ/kg)	12.30	12.27	12.22	12.18	12.15
CP (g/kg)	202.7	223.8	262.5	288.0	313.5
Crude fibre (g/kg)	28.20	28.50	29.06	29.43	29.80
Ca (g/kg)	10.00	10.00	10.00	10.00	10.00
Available Phosphorus (g/kg)	5.00	5.00	5.00	5.00	5.00
Na (g/kg)	1.60	1.60	1.60	1.60	1.60
Cl (g/kg)	1.70	1.65	1.60	1.60	1.60
K (g/kg)	6.10	6.86	8.26	9.18	10.10
Mn (mg/kg)	77.08	77.38	77.93	78.30	78.66
Zn (mg/kg)	72.38	73.71	76.17	77.78	79.39
Indispensable amino acids					
Arginine (g/kg)[3]	8.70	9.97	12.32	13.86	15.40
Cystine (g/kg)[3]	3.20	3.54	4.17	4.59	5.00
Isoleucine (g/kg)[3]	7.40	8.14	9.51	10.40	11.30
Lysine (g/kg)[3]	10.20	11.26	13.22	14.51	15.80
Methionine (g/kg)[3]	3.90	4.32	5.09	5.59	6.10
Phenylalanine (g/kg)[3]	7.90	8.76	10.33	11.37	12.40
Threonine (g/kg)[3]	6.20	6.87	8.09	8.90	9.70
Tryptophan (g/kg)[3]	2.30	2.59	3.11	3.46	3.80
Valine (g/kg)[3]	8.20	8.98	10.41	11.36	12.30
Dispensable					
Tyrosine (g/kg)[3]	7.10	7.86	9.26	10.18	11.10

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 31: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 8-12 weeks of age.

Ingredients	Crude protein concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72% - CP)	10.00	13.80	20.80	25.40	30.00
Soybean Meal - (48% - CP)	94.0	129.4	194.6	237.5	280.3
Wheat, White	777.0	735.7	659.6	609.6	559.6
Wheat Bran	25.00	20.25	11.50	5.75	0.00
Corn gluten meal - (60% - CP)	0.00	3.80	10.80	15.40	20.00
Casein	20.00	21.90	25.40	27.70	30.00
Soybean OiL	23.60	25.23	28.24	30.22	32.20
L-Lysine HCl	3.20	3.01	2.66	2.43	2.20
DL-Methionine	2.40	2.67	3.16	3.48	3.80
L-Threonine	1.80	2.22	2.99	3.49	4.00
Common Salt	1.50	1.50	1.50	1.50	1.50
Limestone	7.80	7.31	6.40	5.80	5.20
Dicalcium phosphate	20.00	19.53	18.65	18.08	17.50
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME (MJ/kg)	12.58	12.57	12.56	12.56	12.55
CP (g/kg)	169.3	187.5	220.9	242.9	264.9
Crude fibre (g/kg)	30.10	29.85	29.40	29.10	28.80
Ca (g/kg)	8.50	8.50	8.50	8.50	8.50
Available Phosphorus (g/kg)	4.20	4.20	4.20	4.20	4.20
Na (g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.00	1.98	1.95	1.92	1.90
K (g/kg)	5.60	6.11	7.06	7.68	8.30
Mn (mg/kg)	77.17	77.17	77.17	77.17	77.17
Zn (mg/kg)	70.67	71.56	73.20	74.27	75.35
Indispensable amino acids					
Arginine (g/kg)[3]	6.80	7.85	9.77	11.04	12.30
Cystine (g/kg)[3]	3.00	3.32	3.92	4.31	4.70
Isoleucine (g/kg)[3]	6.10	6.77	7.99	8.80	9.60
Lysine (g/kg)[3]	8.70	9.61	11.29	12.40	13.50
Methionine (g/kg)[3]	3.60	3.96	4.63	5.06	5.50
Phenylalanine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Threonine (g/kg)[3]	5.30	5.83	6.81	7.46	8.10
Tryptophan (g/kg)[3]	1.90	2.13	2.55	2.82	3.10
Valine (g/kg)[3]	6.50	7.26	8.66	9.58	10.50
Dispensable					
Tyrosine (g/kg)[3]	5.80	6.48	7.74	8.57	9.40

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 32: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 12-16 weeks of age.

Ingredients	Crude protein concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal (72% CP)	0.00	8.13	23.11	32.96	42.80
Soybean Meal –(48% CP)	82.0	99.9	132.8	154.4	176.0
Wheat, White	718.6	707.4	686.7	673.2	659.6
Wheat Middlings	40.00	32.40	18.40	9.20	0.00
Wheat Bran	50.00	40.50	23.00	11.50	0.00
Corn gluten meal, (60% CP)	0.00	1.90	5.40	7.70	10.00
Casein	0.00	5.70	16.20	23.10	30.00
Soybean OiL	57.90	53.76	46.13	41.11	36.10
L-Lysine HCl	4.90	4.63	4.14	3.82	3.50
DL-Methionine	3.00	3.29	3.81	4.16	4.50
L-Threonine	2.60	2.83	3.25	3.52	3.80
Common Salt	1.60	1.54	1.44	1.37	1.30
Limestone	7.70	6.66	4.73	3.47	2.20
Dicalcium phosphate	18.00	17.72	17.19	16.85	16.50
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
ME (MJ/kg)	12.97	12.97	12.96	12.95	12.95
CP (g/kg)	146.1	161.9	190.9	209.9	229.0
Crude fibre (g/kg)	33.60	32.46	30.36	28.98	27.60
Ca (g/kg)	7.50	7.50	7.50	7.50	7.50
Available Phosphorus (g/kg)	3.80	3.80	3.80	3.80	3.80
Na (g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.35	2.34	2.24	2.20	2.17
K (g/kg)	5.70	5.87	6.19	6.39	6.60
Mn (mg/kg)	81.79	80.53	78.22	76.69	75.17
Zn (mg/kg)	75.37	75.07	74.51	74.15	73.78
Indispensable amino acids					
Arginine (g/kg)[3]	5.90	6.68	8.11	9.06	10.00
Cystine (g/kg)[3]	2.80	3.10	3.66	4.03	4.40
Isoleucine (g/kg)[3]	4.70	5.35	6.54	7.32	8.10
Lysine (g/kg)[3]	8.10	8.96	10.53	11.57	12.60
Methionine (g/kg)[3]	3.60	3.98	4.68	5.14	5.60
Phenylalanine (g/kg)[3]	5.20	5.90	7.20	8.05	8.90
Threonine (g/kg)[3]	5.20	5.77	6.82	7.51	8.20
Tryptophan (g/kg)[3]	1.60	1.77	2.09	2.29	2.50
Valine (g/kg)[3]	4.90	5.70	7.17	8.13	9.10
Dispensable					
Tyrosine (g/kg)[3]	4.50	5.15	6.34	7.12	7.90

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 33: Ingredient and nutrient composition of experimental diets with different protein concentration used for turkeys for growth phase from 16-20 weeks of age.

Ingredients	Crude protein concentration (% of the commercial recommendations)				
	77-T1	85-T2	100-T3	110-T4	120-T5
			g/kg		
Fish meal - (72%- CP)	0.00	5.70	16.20	23.10	30.00
Soybean Meal - (48%- CP)	42.0	57.4	85.7	104.4	123.0
Wheat, White	781.1	770.4	750.6	737.6	724.6
Wheat Bran	68.00	55.08	31.28	15.64	0.00
Corn gluten meal - (60%-CP)	0.00	1.90	5.40	7.70	10.00
Casein	0.00	5.70	16.20	23.10	30.00
Soybean OiL	65.00	61.28	54.42	49.91	45.40
L-Lysine HCl	3.40	2.98	2.21	1.71	1.20
DL-Methionine	1.90	2.05	2.33	2.52	2.70
L-Threonine	1.00	1.06	1.16	1.23	1.30
Common Salt	1.60	1.54	1.44	1.37	1.30
Limestone	6.80	6.04	4.64	3.72	2.80
Dicalcium phosphate	15.50	15.22	14.69	14.35	14.00
Vit/min Premix[1]	3.20	3.20	3.20	3.20	3.20
Coccidiostat	0.50	0.50	0.50	0.50	0.50
Pellet binder	10.00	10.00	10.00	10.00	10.00
Calculated nutrient analysis					
M.E. (MJ/kg)	13.39	13.39	13.38	13.38	13.38
CP (g/kg)	127.1	140.6	165.4	181.8	198.1
Crude fibre (g/kg)	32.90	31.86	29.93	28.67	27.40
Ca (g/kg)	6.50	6.50	6.50	6.50	6.50
Available Phosphorus (g/kg)	3.20	3.20	3.20	3.20	3.20
Na (g/kg)	1.20	1.20	1.20	1.20	1.20
Cl (g/kg)	2.10	2.01	2.00	1.93	1.83
K (g/kg)	5.00	5.15	5.43	5.62	5.80
Mn (mg/kg)	78.14	77.22	75.52	74.40	73.28
Zn mg/kg	71.07	70.97	70.79	70.68	70.56
Indispensable amino acids					
Arginine (g/kg)[3]	4.80	5.48	6.74	7.57	8.40
Cystine (g/kg)[3]	2.30	2.55	3.00	3.30	3.60
Isoleucine (g/kg)[3]	4.20	4.79	5.87	6.59	7.30
Lysine (g/kg)[3]	6.00	6.63	7.78	8.54	9.30
Methionine (g/kg)[3]	2.80	3.07	3.56	3.88	4.20
Phenylalanine (g/kg)[3]	4.60	5.23	6.38	7.14	7.90
Threonine (g/kg)[3]	3.60	3.98	4.68	5.14	5.60
Tryptophan (g/kg)[3]	1.40	1.53	1.78	1.94	2.10
Valine (g/kg)[3]	4.30	5.02	6.35	7.23	8.10
Dispensable					
Tyrosine (g/kg)[3]	4.00	4.59	5.67	6.39	7.10

[1]The vitamin and mineral premix (Target Feeds Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME values of the diets were calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 34: Analysed composition of experimental diets for 4-8 wks growth phase.

Determined values	Crude protein concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	866.9	866.9	866.9	866.9	866.9
Crude protein (g/kg)	180.7	200.8	238.0	262.0	286.3
Gross energy (MJ/kg)	16.71	16.75	16.86	16.90	16.96
Ash (g/kg)	64.94	65.75	67.23	68.21	69.19
Crude fat (g/kg)	36.93	38.42	41.15	42.94	44.74
Neutral detergent fibre (g/kg)	53.93	54.09	54.40	54.59	54.79
Ca (g/kg)	9.36	9.78	10.53	11.03	11.53
Total Phosphorous (g/kg)	7.49	7.85	8.50	8.93	9.36
Na (g/kg)	1.56	1.58	1.61	1.63	1.65
K (g/kg)	6.76	7.65	9.29	10.37	11.44
Cu (mg/kg)	18.81	19.13	19.70	20.08	20.46
Mg (g/kg)	1.30	1.42	1.63	1.77	1.91
Mn (mg/kg)	110.1	119.7	137.3	148.8	160.4
Zn (mg/kg)	184.7	173.3	152.4	138.6	124.9
Indispensable amino acids					
Arginine (g/kg)	6.94	8.46	11.25	13.08	14.91
Histidine (g/kg)	2.61	3.20	4.28	4.99	5.70
Isoleucine (g/kg)	7.10	8.22	10.29	11.65	13.01
Leucine (g/kg)	11.86	13.68	17.04	19.25	21.46
Lysine (g/kg)	8.27	9.82	12.67	14.54	16.41
Methionine (g/kg)	3.21	3.59	4.30	4.77	5.24
Phenylalanine (g/kg)	7.65	8.73	10.72	12.03	13.33
Threonine (g/kg)	5.01	6.39	8.93	10.61	12.28
Valine (g/kg)	8.22	9.24	11.12	12.36	13.59
Dispensable					
Alanine (g/kg)	5.70	6.78	8.77	10.08	11.38
Aspartic acid (g/kg)	12.81	15.40	20.17	23.31	26.45
Glutamic acid (g/kg)	36.35	40.11	47.03	51.58	56.13
Glycine (g/kg)	5.46	6.41	8.15	9.30	10.44
Serine (g/kg)	5.18	6.11	7.83	8.95	10.07
Tyrosine (g/kg)	3.80	4.46	5.67	6.47	7.27

Table 35: Analysed composition of experimental diets for 8-12 wks growth phase.

Determined values	Crude protein concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	844.7	844.9	845.2	845.5	845.7
Crude protein (g/kg)	151.3	171.8	209.6	234.4	259.5
Gross energy (MJ/kg)	16.10	16.22	16.46	16.61	16.78
Ash (g/kg)	5.05	5.19	5.45	5.61	5.79
Crude fat (g/kg)	43.10	43.99	45.65	46.74	47.88
Ca (g/kg)	9.46	9.46	9.46	9.46	9.48
Total Phosphorous (g/kg)	6.70	6.86	7.15	7.34	7.54
Na (g/kg)	0.93	1.01	1.16	1.25	1.35
K (g/kg)	5.75	6.37	7.53	8.28	9.05
Cu (mg/kg)	18.59	17.84	16.45	15.53	14.64
Mg (g/kg)	1.18	1.28	1.46	1.57	1.69
Mn (mg/kg)	109.0	118.0	134.6	145.4	156.5
Zn (mg/kg)	130.1	128.9	126.5	124.9	123.5
Indispensable amino acids					
Arginine (g/kg)	6.25	7.49	9.78	11.28	12.79
Histidine (g/kg)	2.62	3.23	4.35	5.09	5.84
Isoleucine (g/kg)	6.46	7.34	8.96	10.02	11.09
Leucine (g/kg)	10.84	12.49	15.53	17.52	19.54
Lysine (g/kg)	9.10	9.90	11.37	12.34	13.32
Methionine (g/kg)	3.00	3.65	4.84	5.62	6.41
Phenylalanine (g/kg)	6.90	7.98	9.97	11.27	12.59
Threonine (g/kg)	5.16	6.23	8.20	9.50	10.80
Valine (g/kg)	6.88	7.77	9.41	10.49	11.58
Dispensable					
Alanine (g/kg)	5.20	5.83	6.99	7.75	8.52
Aspartic acid (g/kg)	11.69	13.47	16.75	18.90	21.08
Glutamic acid (g/kg)	33.19	36.39	42.29	46.16	50.09
Glycine (g/kg)	4.85	6.02	8.18	9.60	11.03
Serine (g/kg)	4.78	5.42	6.59	7.35	8.13
Tyrosine (g/kg)	3.52	4.22	5.51	6.36	7.22

Table 36: Analysed composition of experimental diets for 12-16 wks growth phase.

Determined values	Crude protein concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	847.1	848.90	849.5	850.6	851.6
Crude protein (g/kg)	144.3	159.3	186.9	205.0	223.2
Gross energy (MJ/kg)	16.92	16.93	16.93	16.93	16.93
Ash (g/kg)	48.02	48.85	50.40	51.40	52.40
Crude fat (g/kg)	71.23	67.97	61.94	57.94	53.93
Ca (g/kg)	8.22	8.42	8.80	9.04	9.29
Total Phosphorous (g/kg)	6.67	6.85	7.18	7.40	7.62
Na (g/kg)	0.76	0.83	0.95	1.03	1.11
K (g/kg)	6.01	6.31	6.86	7.22	7.58
Cu (mg/kg)	29.14	27.22	23.69	21.35	19.00
Mg (g/kg)	1.44	1.44	1.45	1.45	1.45
Mn (mg/kg)	118.6	120.7	124.5	127.0	129.5
Zn (mg/kg)	105.9	110.0	117.7	122.8	127.8
Indispensable amino acids					
Arginine (g/kg)	6.77	7.37	8.50	9.24	9.98
Histidine (g/kg)	2.67	2.96	3.51	3.87	4.23
Isoleucine (g/kg)	6.51	7.11	8.23	8.96	9.69
Leucine (g/kg)	11.03	12.04	13.91	15.14	16.37
Lysine (g/kg)	9.16	9.90	11.27	12.17	13.07
Methionine (g/kg)	3.62	3.99	4.69	5.15	5.61
Phenylalanine (g/kg)	7.34	7.90	8.92	9.59	10.27
Threonine (g/kg)	5.34	6.03	7.30	8.13	8.96
Valine (g/kg)	6.88	7.49	8.62	9.37	10.11
Dispensable					
Alanine (g/kg)	5.10	5.68	6.74	7.44	8.15
Aspartic acid (g/kg)	12.25	13.43	15.62	17.06	18.51
Glutamic acid (g/kg)	36.45	38.71	42.89	45.63	48.38
Glycine (g/kg)	4.70	5.51	7.01	7.99	8.98
Serine (g/kg)	4.48	4.98	5.91	6.51	7.12
Tyrosine (g/kg)	4.12	4.43	5.01	5.38	5.76

Table 37: Analysed composition of experimental diets for 16-20 wks growth phase.

Determined values	Crude protein concentration (% of the commercial recommendations)				
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**
Dry matter (g/kg)	842.8	844.0	846.3	847.7	849.2
Crude protein (g/kg)	120.0	134.1	160.0	177.2	194.3
Gross energy (MJ/kg)	16.86	16.88	16.91	16.95	16.96
Ash (g/kg)	43.25	43.62	44.31	44.81	45.25
Crude fat (g/kg)	76.38	72.65	65.77	61.29	56.71
Ca (g/kg)	7.59	7.71	7.93	8.09	8.24
Total Phosphorous (g/kg)	6.11	6.29	6.61	6.83	7.04
Na (g/kg)	0.84	0.88	0.94	0.98	1.02
K (g/kg)	4.81	5.12	5.69	6.07	6.45
Cu (mg/kg)	15.76	16.22	17.05	17.62	18.17
Mg (g/kg)	1.10	1.13	1.19	1.23	1.27
Mn (mg/kg)	123.1	123.7	124.9	125.8	126.5
Zn (mg/kg)	107.9	109.8	113.3	115.7	118.0
Indispensable amino acids					
Arginine (g/kg)	4.28	4.93	6.13	6.92	7.71
Histidine (g/kg)	1.96	2.23	2.73	3.06	3.40
Isoleucine (g/kg)	4.88	5.63	7.01	7.93	8.85
Leucine (g/kg)	8.61	9.94	12.41	14.05	15.68
Lysine (g/kg)	7.17	7.89	9.22	10.10	10.98
Methionine (g/kg)	2.86	3.09	3.52	3.80	4.08
Phenylalanine (g/kg)	5.95	6.69	8.06	8.96	9.87
Threonine (g/kg)	2.71	3.23	4.20	4.84	5.48
Valine (g/kg)	5.45	6.19	7.58	8.49	9.41
Dispensable					
Alanine (g/kg)	3.40	4.00	5.13	5.87	6.61
Aspartic acid (g/kg)	8.53	9.97	12.64	14.41	16.17
Glutamic acid (g/kg)	30.54	33.24	38.22	41.55	44.84
Glycine (g/kg)	3.68	4.29	5.43	6.19	6.94
Serine (g/kg)	2.47	3.08	4.21	4.96	5.71
Tyrosine (g/kg)	2.44	2.93	3.82	4.42	5.01

4.2.2 Statistical procedure

Similar as explained in Section 3.2.14 of Chapter 3, however, treatment factor i.e. nutrient density is replaced with dietary crude protein (CP) concentration.

4.3 **Results**

The analysed chemical composition of the basal diets is presented in Table 34 to Table 37. The analysed values for the concentration of crude protein (CP) content was lower than the calculated values in tables (Table 30 to Table 33), however, analysed values for K, Ca, Na, Mn and Zn concentration were generally higher than the calculated values.

Beside other factors (explained in Section 3.3 Chapter 3) since calculated and analysed values for amino acids were on a digestible and total concentration basis therefore, no comparison was done for amino acid concentration.

4.3.1 Water intake measurements and Litter quality associated parameters

Increased dietary crude protein (CP) concentration had a positive effect on water intake (WI) and water:feed (W:F) which described ina linear way (P<0.001) as the CP concentration increased (Table 43). However, there was no effect (P>0.05) of dietary treatments noted for feed intake for water:feed (FI W:F). There was a significant effect (P<0.001) of time on these parameters, where an increase (P<0.001) in WI (Figure 39 and Figure 40) and FI W:F was noted as the birds aged. On the contrary W:F linearly decreased (P<0.001) with the increase of the age of the birds. There was a significant interaction (P<0.05) between dietary treatments by time period for W:F (Table 43). The response of the W:F was a positive linear function (P<0.001) of dietary CP concentration from 8-20 weeks, while it was best described as quadratic response from 4-8 weeks. Contrast tests revealed a significantly higher WI (P<0.05) and W:F (P<0.01) for control diet fed birds when compared with birds fed lower CP concentration diets (Table 43). There was no difference (P>0.05) between control fed birds and birds fed higher CP concentration diets for WI and W:F (Table 43).

Increased dietary CP concentration had a positive linear effect (P<0.001) on litter moisture (LM), litter NH_3 and litter score (LS) which increased linearly as the dietary CP increased (Table 38 and Figure 35). Increased dietary CP tended to have a positive effect on litter pH which tended to increase linearly (P=0.09) with the increase in dietary CP concentration. LM and LS linearly increased (P<0.001) with the increase of the age of the birds, the highest LM (Figure 33) and LS (Figure 37) values were observed during the last feeding phases of the study. The time response of litter NH_3 concentration and pH was quadratic (P<0.01) as the highest values were observed for the second (8-12 week) and third (12-16 week) growing phases (for NH_3 Figure 36). The results for LM (P<0.01), litter NH_3 (P<0.001), litter pH (P<0.05) and LS (P<0.05) were subject to a dietary CP concentration x time interaction, showing that there were different patterns of response during different growing phases. For example, the response of the LS to diets T4 and T5 seems not to be influenced by the feeding phase although the response of feeding the rest of the diets tended to follow a quadratic pattern (Table 38 and Figure 38). Likewise, the response of the LM to diets T3, T4 and T5 seems not to be influenced by the feeding phase although the response of feeding the rest of the diets (T1 and T2) tended to follow a quadratic pattern (Table 38 and Figure 34). The response of litter NH_3 (Figure 36) and pH to dietary CP concentration during different feeding phases was also inconsistent.

Notably litter temperature (T°) was not affected by dietary density (P>0.05) but responded in a quadratic manner to time as the lowest T° was observed between 8-12 weeks of age. The comparison contrast test finds significantly (P<0.001) higher LM, NH_3 and LS in groups fed control diet when compared with groups fed lower CP concentration diets, whereas, the comparison of two groups for pH and T° was not significantly different (P>0.05). Similarly, no difference (P>0.001) was recorded when control diet fed group was compared with higher nutrient density fed groups for pH and T°. However, significantly (P<0.001) higher LM, NH_3 and LS was recorded in groups fed higher dietary concentration diets when compared with control diet (Table 38).

Correlations analysis (Table 47) reveled that LM was associated with parameters such as weight gain (r = 0.996; P<0.001), litter scores (r = 0.993; P<0.001), water to feed ratio (r = 0.991; P<0.001) and water intake (r = 0.977; P<0.001).

4.3.2 Leg health parameters

As dietary CP increased so did the prevalence of hock burn (HB) (P<0.05). Increasing dietary CP had a negative linear effect (P<0.01) on good hock scores (GHS), however, resulted in a linear increase in bad hock scores (BHS) and total hock scores (THS, Figure 29) (P<0.01). The growth phases had a significant effect (P<0.001) on all hock score parameters, where GHS increase with growth phases, conversely BHS and THS (Figure 30) decrease with the progress in growth phases (Table 39). There was no significant (P>0.05) time and diets interaction observed for HB parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower or higher nutrient densities revealed no difference (P>0.05). Almost similar findings were recorded for footpad quality, decreasing dietary CP concentration had a positive linear effect on the health of good footpad condition with linear increase in number good footpad scores (GFPS) (P<0.001). On the contrary occurrence of bad footpad scores (BFPS) and total footpad score (TFPS) increased linearly (P<0.001) with the increase in dietary CP concentration. The growth phases had no significant effect (P>0.05) on all footpad quality parameters (Table 40) i.e. GFPS, BFPS and TFPS (Figure 31 and Figure 32). There was no (P>0.05) time by diets interaction noted for footpad quality parameters. Likewise, comparison of control diet fed birds with groups fed diets with lower dietary CP revealed no difference (P>0.05). However, control diet fed birds had higher GFPS (P<0.05) and lower BFPS (P<0.05) when compared with groups of birds fed higher CP concentration diets.

As with HB characterised as good and bad scores, the results obtained using GLMM showed an increase in the HB incidence in birds fed diet containing higher CP concentrations (P<0.05) - an average of 178% higher HB incidence for groups fed 110

and 120% CP concentration diets in comparison to the groups fed diets containing 77 and 85% CP concentration (Table 41). However, there was a significant decrease (P<0.001) in the incidence of HB as bird grew older 45% vs. 9.26% birds with HB>0 at the end of week 8 and 20, respectively. Likewise, the incidence of footpad dermatitis (FPD) was more frequent in birds fed diet containing higher CP concentrations (P<0.01) - an average of 340% higher FPD incidence for groups fed 110 and 120% CP concentration diets in comparison to the groups fed diets containing 77 and 85% CP concentration (Table 41). Whereas, the effect of time period was not significant (P>0.05) higher incidences were recorded at the end of week 12 (14.60% birds with FPD>0) which fell at the end of week 16 (7.93% birds with FPD>0) and increase again at week 20 (9.70% birds with FPD>0).

Correlations between variables are shown in (Table 47). Hock burn score (HBS) was associated with many of the parameters and in particular litter moisture (r = 0.971; P<0.001), weight gain (r = 0.970; P<0.001), litter scores (r = 0.952; P<0.001), water to feed ratio (r = 0.938; P<0.001), water intake (r = 0.916; P<0.001) and ammonia in litter (r = 0.873; P<0.001). Interestingly, footpad score (FPS) was also associated with litter scores (r = 0.999; P<0.001), weight gain (r = 0.996; P<0.001), litter moisture (r = 0.990; P<0.001), water intake (r = 0.985; P<0.001), water to feed ratio (r = 0.977; P<0.001) as well as with hock score (r = 0.946; P<0.001).

4.3.3 Growth performance, dietary nutrient intake and utilisation

Overall body weight (BW) was higher than the breed standards at 20 weeks of age, 18.38 kg vs. target of 15.18 kg (Data not included in tables). Increasing dietary CP had a positive linear effect on total weight gain (TWG, P<0.001), weight gain (WG, P<0.001) and feed conversion efficiency (FCE, P<0.05) (Table 42). The response of feed intake (FI) was best described as a quadratic function (P<0.01), and there was no effect (P>0.05) of dietary treatments on protein efficiency ratio (PER). There was a significant effect (P<0.001) of time on these parameters so there was an increase in TWG, WG, FI and FCE, but a reduction in PER as bird aged. There was a significant interaction (P<0.05) between dietary CP concentration by time period for parameters TWG, WG, and FCE (P<0.001) (Table 42). For example, the response of the TWG and WG to diet T1 was not influenced by the feeding phase although the response of feeding the rest of the diets (T2, T3, T4 and T5) tended to follow a quadratic pattern (Table 42). Likewise, the response of the FCE to diets T3, T4 and T5 was not influenced by the feeding phase however, the response of feeding the rest of the diets (T1 and T2) tended to follow a quadratic pattern (Table 42). Comparison of control diet fed birds with groups of birds fed lower CP concentration diets revealed a significantly (P<0.01 and P<0.05) higher TWG, WG and FI for control diet fed birds over lower CP concentration fed birds. In contrast control diet fed

birds had significantly (P<0.05) lower TWG, WG when compared with birds that were fed higher dietary CP concentration diets (Table 42). Notably there was no difference (P>0.05) in FCE or PER between the control diet fed birds and either of the lower or higher CP concentration diets fed birds. Similarly there was no difference (P>0.05) in FI between the control diet fed birds and birds fed a higher CP concentration diets (Table 42).

The response of apparent metabolisable energy (AME), apparent metabolisable energy nitrogen corrected (AMEn), apparent metabolisable energy intake (AME I), crude protein digestibility (CPD), dry matter digestibility (DMD) and organic matter digestibility (OMD) (Table 44) to dietary CP was best described as quadratic (P<0.05) (Table 44). Overall, control birds had a lower (P<0.001) ME:CP when compared to birds offered the lower CP concentration diets (77 and 85% of breed standard recommendations) – almost 22% lower. However, ME:CP was lower for the control group compared with birds fed diets containing higher CP concentrations (110 and 120% of breed standard recommendations) - almost 13% lower, in the case of control diet fed birds. No difference (P>0.05) existed for the AME, AMEn, AME I, DMD and OMD when comparisons were made between control diet and lower CP diet fed birds. Similarly, no difference (P>0.05) existed in AME, AMEn, CPD, DMD and OMD when compared with birds fed diets with higher CP concentrations (Table 44).

As dietary CP concentration increased there was a linear increase (P<0.001) in nitrogen excretion (NEx), nitrogen excretion as part of amino acids (AAN), nitrogen excretion as uric acid (UAN), and neutral detergent fibre fraction intake (NDF I). In contrast energy efficiency ratio (EER) had a quadratic response (P<0.05) to dietary CP concentration. A significantly higher (P<0.01) NEx, AAN and UAN was noted when control diet fed birds were compared with lower CP concentration fed birds (Table 44). However, values for NEx were lower (P<0.001) when control fed birds were compared with groups fed with diets containing higher CP concentrations. Whereas, the difference for AAN, UAN was not significant (P>0.05) (Table 44) when comparisons were made between the birds fed the control diet and those fed diets with higher CP concentrations. There was no treatment difference (P>0.05) in EER and NDF I (Table 44).

Overall, there was a positive linear response of digestibility of some amino acids i.e. Ala, Arg, Glu, Ser, Thr to dietary protein concentration. However, the digestibility of amino acids such as Asp, Ile, Leu, Lys, Phe, Tyr and Val was described best as quadratic (P<0.05) to dietary protein concentration (Table 45). No differences (P>0.05) in amino acid digestibilities were noted when control diet fed groups were compared with lower or higher protein diet fed groups. The only exception of significant difference (P<0.05) in

comparison of control with high protein fed birds was that of Asp digestibility which was higher in case of higher protein concentration fed groups.

Overall response of minerals digestibility to dietary protein concentration (during digestibility measurements after 7th week at 49 days of age) i.e. for Ca, Cu, K, Mg, Mn and P was best described as quadratic ($P<0.001$) (Table 46). Whereas, the response of Cu, Na and Zn digestibilities to dietary CP concentration were best described as a linear function ($P<0.05$ and $P<0.01$). Comparison contrast test revealed no difference ($P<0.05$) when birds fed on control diets were compared with birds fed on either of lower or higher CP concentration diets for Cu, K, Mg, Mn, Na and Zn digestibilities. Similarly birds fed the control diet were not different ($P<0.05$) from higher CP concentration diets for Ca and P digestibilities, however, birds fed lower CP concentration diets had higher ($P>0.05$) digestibilities for same minerals when compared with control diet fed birds (Table 46).

Correlations analysis (Table 47) reveled that CPD was associated positively with DMD ($r = 0.939$; $P<0.001$) and both CPD and DMD were negatively associated with feed intake ($r = -0.993$; $P<0.001$) and ($r = -0.896$; $P<0.001$) respectively (Table 47).

Table 38: Effect of dietary CP concentration and time on litter moisture (LM), litter ammonia (NH_3, ppm), litter pH (pH), litter temperature (T°) and litter score (LS) parameters.

	Treatments		LM	NH_3	pH	T °	LS
	Diets						
	T1		250.4	6.44	7.79	20.65	1.36
	T2		269.3	6.91	7.89	20.41	1.50
	T3		319.3	8.60	8.11	20.34	1.73
	T4		350.1	10.29	8.50	20.50	1.99
	T5		357.1	13.28	8.46	20.34	2.06
SEM			13.02	0.478	0.091	0.171	0.061
	Time (wks)						
	4-8		240.3	3.07	7.69	20.84	1.41
	8-12		320.5	16.27	8.66	19.80	1.88
	12-16		341.2	11.01	8.28	20.37	1.89
	16-20		334.8	6.07	7.97	20.79	1.74
SEM			11.04	0.284	0.055	0.156	0.042
	Diets	**Time (wks)**					
	T1	4-8	222.7	3.13	7.14	21.04	1.20
	T2	4-8	213.1	2.66	7.20	20.87	1.33
	T3	4-8	247.3	2.62	7.64	20.66	1.43
	T4	4-8	275.1	3.79	8.38	20.94	1.56
	T5	4-8	243.1	3.13	8.10	20.66	1.56
	T1	8-12	292.1	13.07	8.49	19.87	1.61
	T2	8-12	295.0	15.14	8.52	19.70	1.51
	T3	8-12	316.1	16.54	8.63	19.49	1.89
	T4	8-12	344.6	16.21	8.76	20.37	2.14
	T5	8-12	354.9	20.36	8.87	19.59	2.26
	T1	12-16	251.9	7.07	7.95	20.60	1.29
	T2	12-16	277.4	7.36	8.15	20.24	1.57
	T3	12-16	358.8	10.50	8.28	20.44	1.99
	T4	12-16	392.1	13.36	8.49	20.24	2.19
	T5	12-16	425.8	16.79	8.52	20.30	2.39
	T1	16-20	234.8	2.50	7.57	21.09	1.34
	T2	16-20	291.6	2.50	7.68	20.81	1.60
	T3	16-20	354.9	4.71	7.89	20.79	1.62
	T4	16-20	388.5	7.79	8.37	20.43	2.09
	T5	16-20	404.4	12.86	8.36	20.83	2.04
SEM			19.96	0.729	0.124	0.347	0.101
	Probabilities of statistical differences						
Diets			<0.001	<0.001	<0.001	NS	<0.001
Linear			<0.001	<0.001	P=0.09	NS	<0.001
Quadratic			NS	<0.05	NS	NS	NS
Contrast 1			<0.001	<0.01	NS	NS	<0.001
Contrast 2			<0.05	<0.001	NS	NS	<0.001
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			<0.01	<0.001	<0.05	NS	<0.05

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 39: Effect of dietary CP concentration and time on leg health parameters i.e. good hock score (GHS), bad hock score (BHS) and total hock score (THS).

	Treatments		GHS	BHS	THS
	Diets				
	T1		0.886	0.114	0.207
	T2		0.857	0.143	0.157
	T3		0.729	0.271	0.482
	T4		0.620	0.380	0.648
	T5		0.634	0.366	0.615
SEM			0.0669	0.0669	0.1481
	Time (wks)				
	4-8		0.550	0.450	0.593
	8-12		0.729	0.271	0.500
	12-16		0.836	0.164	0.299
	16-20		0.866	0.134	0.296
SEM			0.0265	0.0265	0.0514
	Diets	**Time (wks)**			
	T1	4-8	0.657	0.343	0.429
	T2	4-8	0.543	0.457	0.514
	T3	4-8	0.586	0.414	0.586
	T4	4-8	0.429	0.571	0.771
	T5	4-8	0.536	0.464	0.664
	T1	8-12	0.943	0.057	0.171
	T2	8-12	0.943	0.057	0.057
	T3	8-12	0.743	0.257	0.600
	T4	8-12	0.500	0.500	0.900
	T5	8-12	0.514	0.486	0.771
	T1	12-16	0.971	0.029	0.114
	T2	12-16	0.943	0.057	0.057
	T3	12-16	0.800	0.200	0.400
	T4	12-16	0.714	0.286	0.486
	T5	12-16	0.750	0.250	0.436
	T1	16-20	0.971	0.029	0.114
	T2	16-20	1.000	0.000	0.000
	T3	16-20	0.788	0.212	0.340
	T4	16-20	0.836	0.164	0.436
	T5	16-20	0.736	0.264	0.588
SEM			0.0844	0.0844	0.1784
Probabilities of statistical differences					
Diets			<0.05	<0.05	P=0.08
Linear			<0.01	<0.01	<0.01
Quadratic			NS	NS	NS
Contrast 1			P=0.09	P=0.09	NS
Contrast 2			NS	NS	NS
Time			<0.001	<0.001	<0.001
Diets x Time			P=0.06	P=0.06	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 40: Effect of dietary CP concentration and time on leg health parameters i.e. good footpad score (GFPS), bad footpad score (BFPS) and total footpad score (TFPS).

Treatments		GFPS	BFPS	TFPS
Diets				
T1		0.953	0.047	0.047
T2		0.936	0.064	0.100
T3		0.875	0.125	0.166
T4		0.771	0.229	0.260
T5		0.779	0.221	0.271
SEM		0.0357	0.0357	0.0518
Time (wks)				
4-8		--	--	--
8-12		0.824	0.176	0.217
12-16		0.897	0.103	0.109
16-20		0.867	0.133	0.180
SEM		0.0305	0.0305	0.0385
Diets	**Time (wks)**			
T1	4-8	--	--	--
T2	4-8	--	--	--
T3	4-8	--	--	--
T4	4-8	--	--	--
T5	4-8	--	--	--
T1	8-12	0.971	0.029	0.029
T2	8-12	0.907	0.093	0.129
T3	8-12	0.886	0.114	0.143
T4	8-12	0.714	0.286	0.314
T5	8-12	0.643	0.357	0.471
T1	12-16	0.964	0.036	0.036
T2	12-16	0.971	0.029	0.029
T3	12-16	0.914	0.086	0.114
T4	12-16	0.793	0.207	0.207
T5	12-16	0.843	0.157	0.157
T1	16-20	0.924	0.076	0.076
T2	16-20	0.929	0.071	0.143
T3	16-20	0.824	0.176	0.240
T4	16-20	0.807	0.193	0.257
T5	16-20	0.852	0.148	0.183
SEM		0.0662	0.0662	0.0874
Probabilities of statistical differences				
Diets		<0.01	<0.01	<0.05
Linear		<0.001	<0.001	<0.001
Quadratic		NS	NS	NS
Contrast 1		NS	NS	NS
Contrast 2		<0.05	<0.05	NS
Time		NS	NS	NS
Diets x Time		NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 41: Effect of dietary protein concentration and time on leg health parameters i.e. incidences of hock burn (HB) and incidences of footpad dermatitis (FPD), from generalized linear mixed models (GLMM) on logit scale and back transformed on proportion scale (i.e. % of birds with HB>0, FPD>0).

	Treatments	Logit of HB Incidence	Incidence of HB>0	Logit of FPD Incidence	Incidence of FPD>0
	Diets				
	T1	-2.283	9.25	-3.200	3.91
	T2	-2.057	11.33	-2.853	5.45
	T3	-1.287	21.64	-2.075	11.15
	T4	-0.552	36.55	-1.285	21.67
	T5	-0.747	32.15	-1.334	20.84
Min and max SEM		0.4007-0.4648		0.3656-0.5853	
	Time (wks)				
	4-8	-0.211	44.74	--	--
	8-12	-1.158	23.91	-1.766	14.60
	12-16	-1.890	13.13	-2.451	7.93
	16-20	-2.282	9.26	-2.231	9.70
Min and max SEM		0.2324-0.3108		0.3473-0.3856	
Probabilities of statistical differences					
Diets		<0.05		<0.001	
Time		<0.001		NS	

There is a statistical significant difference when P<0.05; SEM- standard errors of means (min= Minimum and max= Maximum). The p-values and SEMs are associated with the estimated means on the logit scale of the analysis.

Table 42: Effect of dietary CP concentration, time (growth phases) and their interaction on total weight gain ((TWG) kg/b/4 weeks), weight gain ((WG) kg/b/d), feed intake ((FI) kg/b/d), feed conversion efficiency ((FCE) wt gain kg/kg FI) and protein efficiency ratio (PER, wt gain kg/CP intake g).

	Treatments		TWG	WG	FI	FCE	PER
	Diets						
	T1		4.01	0.143	0.403	0.394	1.83
	T2		4.13	0.148	0.439	0.382	1.99
	T3		4.36	0.156	0.452	0.396	1.83
	T4		4.61	0.165	0.487	0.393	2.15
	T5		4.63	0.165	0.443	0.418	2.00
SEM			0.069	0.0035	0.0119	0.0075	0.134
	Time (wks)						
	4-8		3.29	0.117	0.202	0.581	2.43
	8-12		4.83	0.172	0.403	0.429	2.05
	12-16		5.05	0.181	0.562	0.326	1.73
	16-20		4.22	0.151	0.612	0.249	1.63
SEM			0.069	0.0025	0.0079	0.0044	0.046
	Diets	**Time (wks)**					
	T1	4-8	2.90	0.104	0.195	0.532	2.19
	T2	4-8	3.09	0.110	0.195	0.566	2.43
	T3	4-8	3.37	0.120	0.201	0.601	2.34
	T4	4-8	3.56	0.127	0.213	0.598	2.75
	T5	4-8	3.52	0.126	0.207	0.610	2.42
	T1	8-12	3.93	0.141	0.349	0.406	1.72
	T2	8-12	4.66	0.166	0.396	0.422	2.09
	T3	8-12	5.03	0.180	0.419	0.432	1.94
	T4	8-12	5.32	0.190	0.441	0.433	2.35
	T5	8-12	5.18	0.185	0.409	0.453	2.16
	T1	12-16	4.92	0.176	0.504	0.363	1.71
	T2	12-16	4.95	0.177	0.570	0.311	1.83
	T3	12-16	4.95	0.177	0.554	0.322	1.55
	T4	12-16	5.31	0.190	0.609	0.314	1.82
	T5	12-16	5.14	0.184	0.575	0.320	1.72
	T1	16-20	4.28	0.153	0.564	0.273	1.69
	T2	16-20	3.82	0.136	0.596	0.229	1.59
	T3	16-20	4.08	0.146	0.636	0.231	1.49
	T4	16-20	4.26	0.152	0.685	0.226	1.68
	T5	16-20	4.68	0.167	0.581	0.287	1.70
SEM			0.150	0.0054	0.0193	0.0113	0.160
	Probabilities of statistical differences						
Diets			<0.001	<0.001	<0.001	<0.05	NS
Linear			<0.001	<0.001	<0.01	<0.05	NS
Quadratic			NS	NS	<0.01	P=0.07	NS
Contrast 1			<0.01	<0.01	<0.05	NS	NS
Contrast 2			<0.01	<0.01	NS	NS	NS
Time			<0.001	<0.001	<0.001	<0.001	<0.001
Diets x Time			<0.05	<0.05	NS	<0.001	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 43: Effect of dietary CP concentration, time (growth phases) and their interaction on water intake ((WI) kg/b/d), feed intake for water ratio feed (FI W:F) kg/b/d) and water ratio feed ((W:F) kg/kg).

Treatments		WI	FI W:F	W:F
Diets				
T1		0.680	0.440	1.62
T2		0.709	0.455	1.64
T3		0.764	0.450	1.83
T4		0.842	0.480	1.91
T5		0.832	0.447	1.95
SEM		0.0266	0.0134	0.041
Time (wks)				
4-8		0.473	0.220	2.15
8-12		0.859	0.427	2.03
12-16		0.843	0.551	1.54
16-20		0.887	0.620	1.44
SEM		0.0141	0.0094	0.031
Diets	**Time (wks)**			
T1	4-8	0.401	0.223	1.80
T2	4-8	0.423	0.216	1.95
T3	4-8	0.471	0.204	2.31
T4	4-8	0.553	0.229	2.42
T5	4-8	0.518	0.229	2.27
T1	8-12	0.713	0.398	1.83
T2	8-12	0.821	0.447	1.84
T3	8-12	0.866	0.424	2.06
T4	8-12	0.946	0.434	2.18
T5	8-12	0.950	0.429	2.23
T1	12-16	0.796	0.554	1.45
T2	12-16	0.790	0.541	1.47
T3	12-16	0.813	0.554	1.49
T4	12-16	0.882	0.559	1.59
T5	12-16	0.934	0.550	1.70
T1	16-20	0.811	0.585	1.40
T2	16-20	0.802	0.617	1.30
T3	16-20	0.907	0.621	1.47
T4	16-20	0.989	0.699	1.44
T5	16-20	0.927	0.580	1.61
SEM		0.0382	0.0226	0.073
Probabilities of statistical differences				
Diets		<0.001	NS	<0.001
Linear		<0.001	NS	<0.001
Quadratic		NS	NS	NS
Contrast 1		<0.05	NS	<0.01
Contrast 2		NS	NS	NS
Time		<0.001	<0.001	<0.001
Diets x Time		NS	NS	<0.05

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 44: The effects of dietary protein on growth performance, water intake, litter quality and nutrient utilisation parameters.

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Energy efficiency ratio (EER, kg/MJ)	0.034	0.039	0.040	0.046	0.031	0.0034	<0.05	NS	<0.05	NS	NS
N Excreted (g/b/d)	2.455	3.354	4.587	5.562	6.219	0.1771	<0.001	<0.001	NS	<0.001	<0.001
AAN (g/b/d)	1.227	1.570	1.748	1.311	1.906	0.0966	<0.001	<0.01	NS	<0.01	NS
UAN (g/b/d)	2.127	3.017	3.814	2.499	4.313	0.2001	<0.001	<0.001	NS	<0.001	NS
NDF I (g/b/d)	9.133	9.142	9.489	10.074	9.818	0.1930	<0.01	<0.001	NS	NS	P=0.065
ME:CP (determined)	0.071	0.060	0.051	0.045	0.045	0.0061	<0.001	<0.001	<0.001	<0.001	<0.01
AME (MJ/kg)	14.78	13.84	14.04	13.54	14.99	0.390	NS	NS	<0.05	NS	NS
AMEn (MJ/kg)	14.61	13.70	13.87	13.39	14.76	0.376	P=0.07	NS	<0.05	NS	NS
AME I (MJ/b/d)	2.473	2.344	2.429	2.484	2.681	0.0690	<0.05	<0.05	<0.05	NS	P=0.082
CPD	0.659	0.556	0.528	0.444	0.570	0.0328	<0.01	<0.01	<0.01	P=0.06	NS
DMD	0.754	0.673	0.680	0.643	0.721	0.0250	<0.05	NS	<0.01	NS	NS
OMD	0.770	0.712	0.712	0.675	0.746	0.0205	<0.05	NS	<0.05	NS	NS

Energy efficiency ratios (EER), N excreted, N excreted as a part of amino acids and uric acid (AAN, UAN), ash digestibility, AME and AMEn (DM basis), crude protein digestibility coefficient (CPD), dry matter digestibility coefficients (DMD) and organic matter digestibility (OMD) were determined at 49th day of age. However, AME I values represents for growth phase 4-8 weeks were obtained on dry matter basis. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 45: The effect of dietary protein on total tract amino acid digestibility coefficients in turkeys at 8 weeks of age.

	Dietary treatments					Probabilities of significant differences					
	77-T1	85-T2	100-T3	110-T4	120-T5	SEM	P	Linear	Quadratic	Contrast 1	Contrast 2
Alanine	0.780	0.771	0.788	0.798	0.866	0.0204	<0.05	<0.01	P=0.06	NS	P=0.093
Arginine	0.866	0.856	0.880	0.882	0.926	0.0114	<0.01	<0.001	P=0.06	NS	NS
Aspartic acid	0.796	0.766	0.798	0.814	0.865	0.0152	<0.01	<0.001	<0.05	NS	<0.05
Glutamic acid	0.890	0.870	0.882	0.880	0.913	0.0089	<0.05	P=0.053	<0.05	NS	NS
Histidine	0.856	0.823	0.870	0.875	0.917	0.0179	<0.05	<0.01	NS	NS	NS
Isoleucine	0.832	0.799	0.824	0.818	0.877	0.0124	<0.01	<0.01	<0.01	NS	NS
Leucine	0.838	0.804	0.830	0.827	0.882	0.0119	<0.01	<0.01	<0.01	NS	NS
Lysine	0.866	0.843	0.872	0.877	0.918	0.0105	<0.001	<0.001	<0.05	NS	P=0.061
Phenylalanine	0.832	0.794	0.822	0.816	0.871	0.0147	<0.05	<0.05	<0.05	NS	NS
Serine	0.847	0.831	0.851	0.857	0.906	0.0154	<0.05	<0.01	P=0.058	NS	NS
Threonine	0.805	0.794	0.834	0.845	0.900	0.0175	<0.01	<0.001	NS	NS	P=0.088
Tyrosine	0.835	0.815	0.840	0.845	0.904	0.0153	<0.01	<0.01	<0.05	NS	P=0.078
Valine	0.808	0.771	0.794	0.783	0.852	0.0153	<0.05	<0.05	<0.01	NS	NS

Amino acids digestibilities were determined at 49th day of age. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 46: The effect of dietary protein on mineral digestibility coefficients in turkeys at 8 weeks of age.

	Dietary treatments					Probabilities of significant differences					
	77-T1	**85-T2**	**100-T3**	**110-T4**	**120-T5**	**SEM**	**P**	**Linear**	**Quadratic**	**Contrast 1**	**Contrast 2**
Ca	0.707	0.491	0.495	0.464	0.587	0.0352	<0.001	<0.05	<0.001	<0.05	NS
Cu	0.113	0.109	0.196	0.220	0.429	0.0708	<0.05	<0.01	NS	NS	NS
K	0.312	0.095	0.149	0.106	0.343	0.0602	<0.05	NS	<0.01	NS	NS
Mg	0.306	0.104	0.193	0.165	0.402	0.0612	<0.05	NS	<0.01	NS	NS
Mn	0.334	0.118	0.226	0.225	0.444	0.0563	<0.01	P=0.065	<0.01	NS	NS
Na	0.406	0.436	0.532	0.567	0.662	0.0597	<0.05	<0.01	NS	NS	NS
P	0.584	0.389	0.423	0.378	0.531	0.0417	<0.01	NS	<0.001	NS	NS
Zn	-0.182	-.0.011	0.096	0.045	0.163	0.0972	NS	<0.05	NS	NS	NS

Minerals digestibilities were determined at 49th day of age. There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 47: Correlation matrix for bird performance, litter quality, dietary nutrient digestibility, and leg health in response changes in dietary CP concentration.

	FI	WG	FCE	WI	W:F	LS	LM	NH_3	CPD	DMD	HBS
WG	0.789										
FCE	-0.001	0.613									
WI	0.855	0.982	0.496								
W:F	0.784	0.981	0.598	0.982							
LS	0.747	0.996	0.656	0.978	0.980						
LM	0.767	0.996	0.641	0.977	0.991	0.993					
NH_3	0.511	0.922	0.833	0.879	0.906	0.951	0.926				
CPD	-0.993	-0.715	0.110	-0.796	-0.718	-0.668	-0.693	-0.413			
DMD	-0.896	-0.458	0.403	-0.589	-0.491	-0.413	-0.437	-0.140	0.939		
HBS	0.734	0.970	0.646	0.916	0.938	0.952	0.971	0.873	-0.656	-0.365	
FPS	0.776	0.996	0.617	0.985	0.977	0.999	0.990	0.938	-0.700	-0.453	0.946

d.f. = 33 Correlation coefficients greater than 0.349 and 0.449 are statistically significant at 5% ($P<0.05$) and 1% level ($P<0.001$), respectively.
Key: FI (feed intake), WG (weight gain), FCE (feed conversion efficiency), WI (water intake), W:F (water to feed ratio), LS (litter score), LM (litter moisture content), NH_3 (ammonia in litter), CPD (crude protein digestibility), DMD (dry matter digestibility), HBS (hock burn scores) and FPS (footpad dermatitis scores).

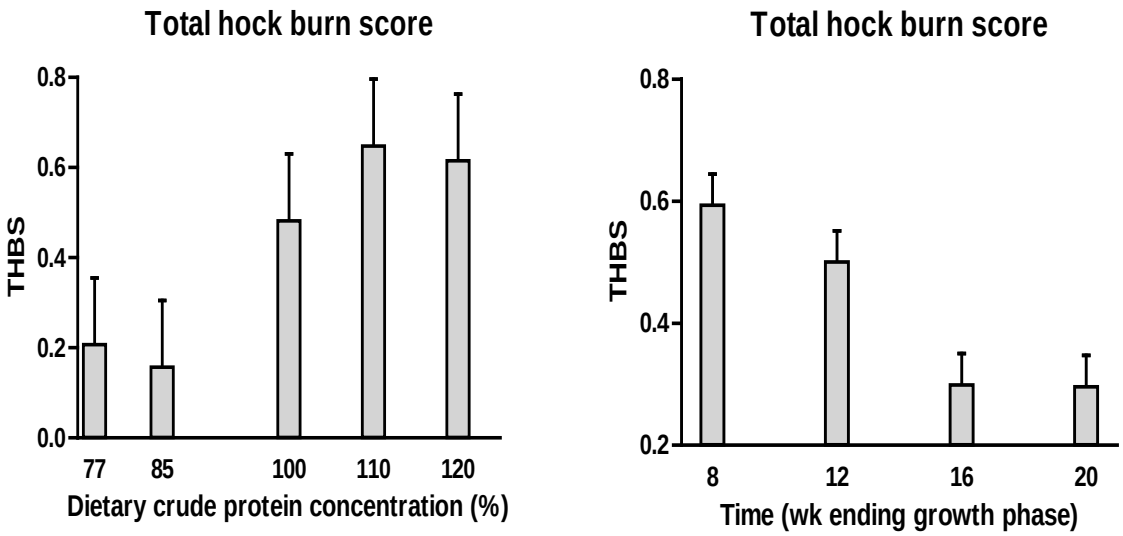


Figure 29: The effect of dietary CP concentration and time (growth phases) on the total hock score (THS) in 20 week old turkeys (error bars represents pooled SEM).

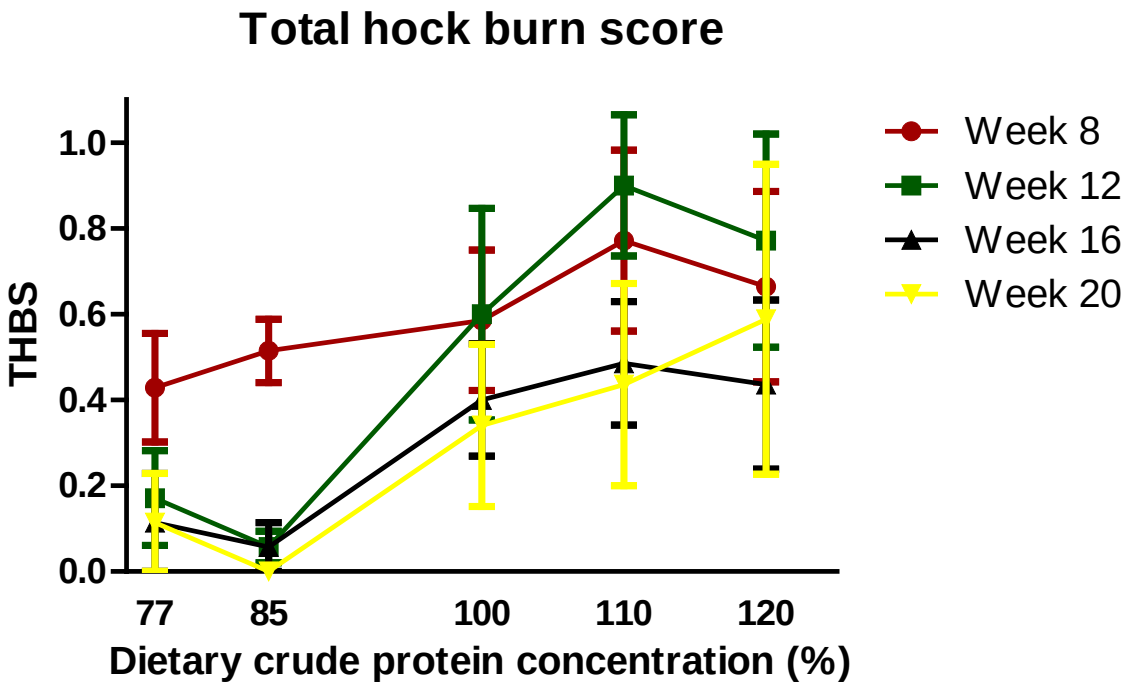


Figure 30: The effect of dietary CP concentration and growth phases on the trend of total hock score (THS) in 20 week old turkeys (SEM bars correspond to each data point).

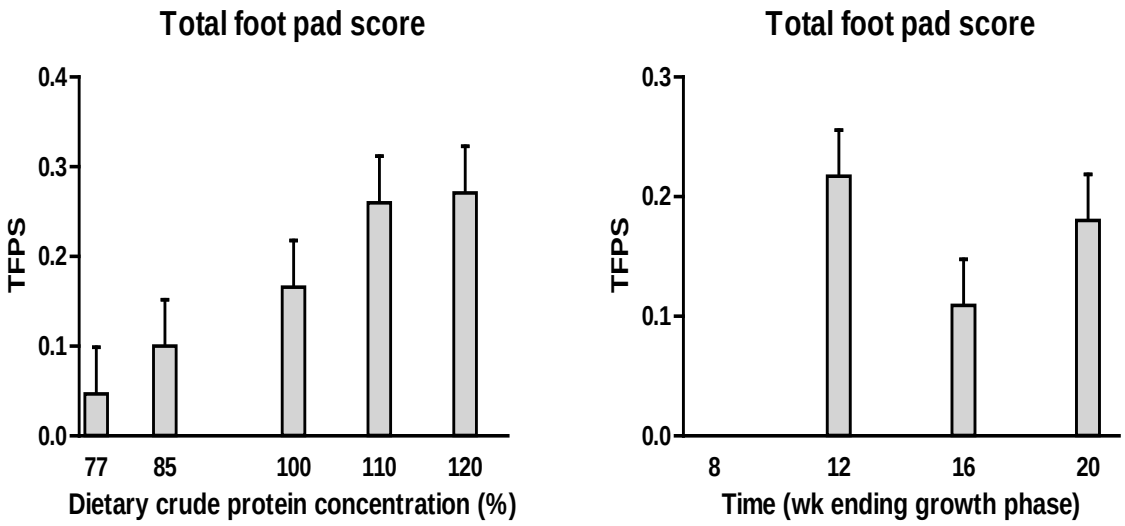


Figure 31: The effect of dietary CP concentration and time (growth phases) on the total foot pad score (TFPS) in 20 week old turkeys (error bars represents pooled SEM).

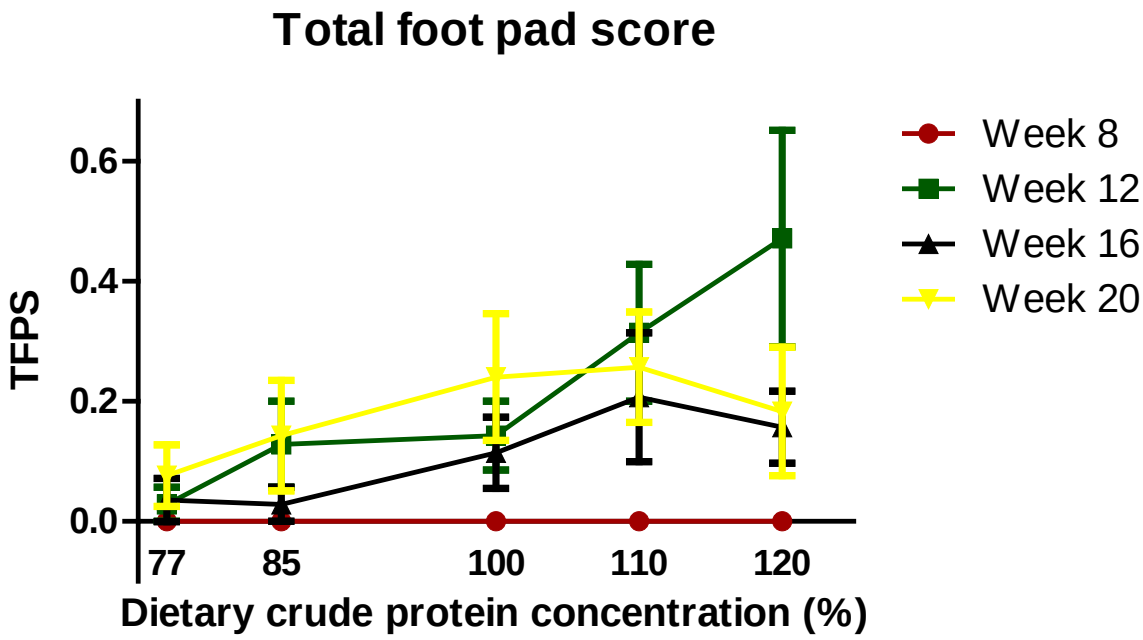


Figure 32: The effect of dietary CP concentration and growth phases on the trend of total foot pad score (TFPS) in 20 week old turkeys (SEM bars correspond to each data point).

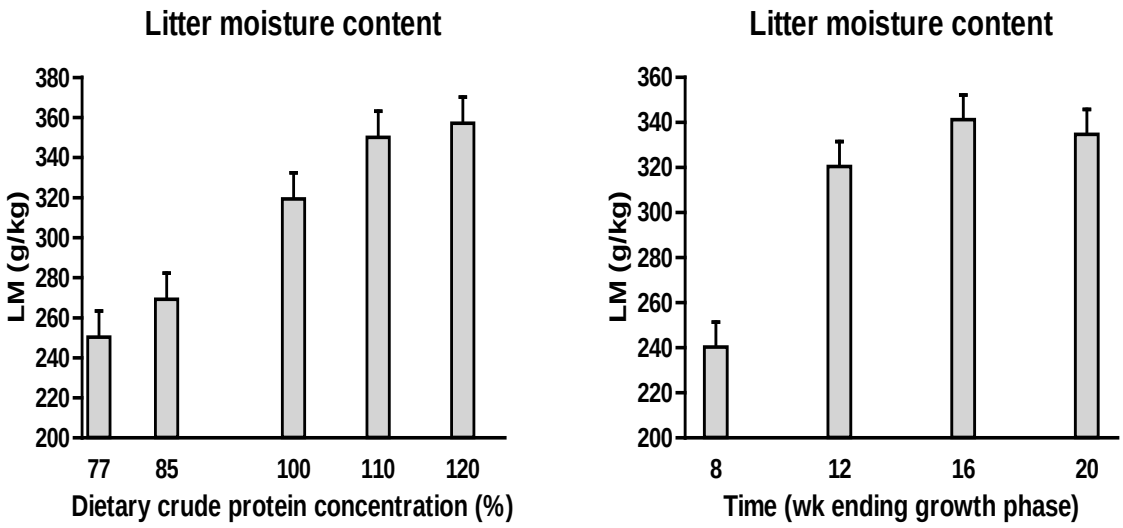


Figure 33: The effect of dietary CP concentration and time (growth phases) on the litter moisture content (LM) in 20 week old turkeys (error bars represents pooled SEM).

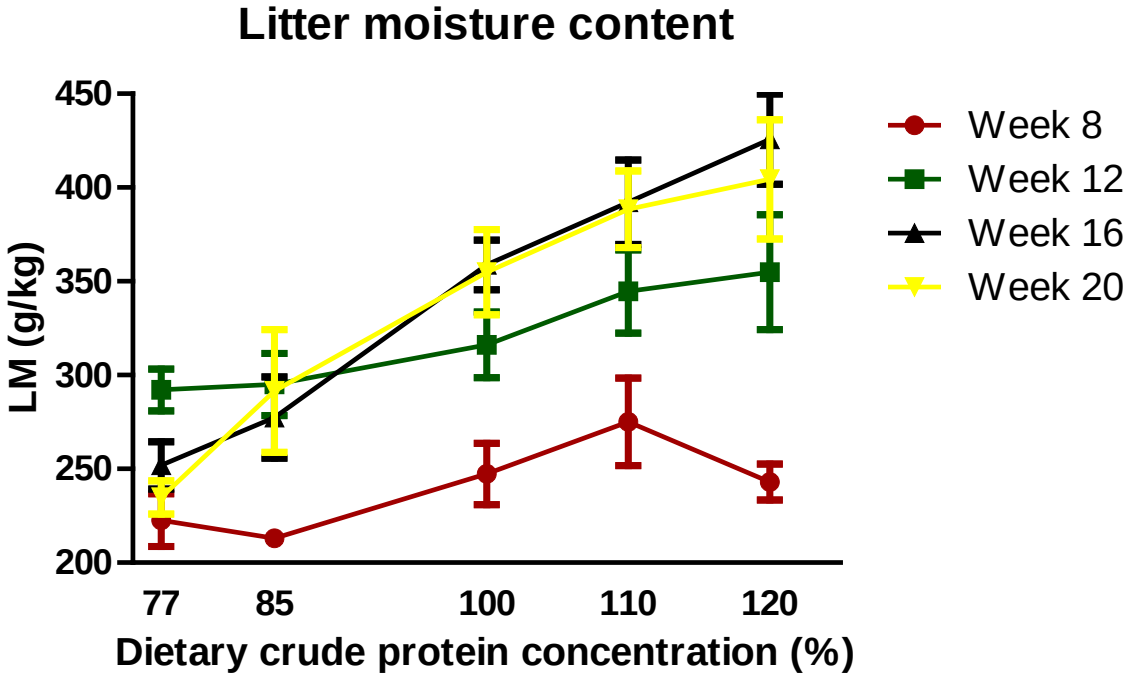


Figure 34: The effect of dietary CP concentration and growth phases on the trend of litter moisture content (LM) in 20 week old turkeys (SEM bars correspond to each data point).

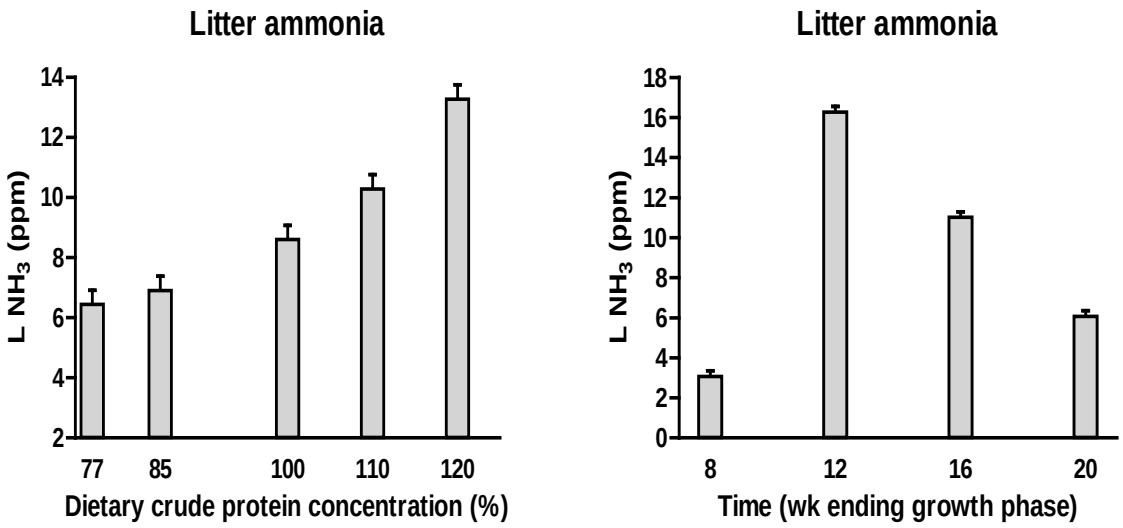


Figure 35: The effect of dietary CP concentration and time (growth phases) on the litter ammonia (L NH_3) in 20 week old turkeys (error bars represents pooled SEM).

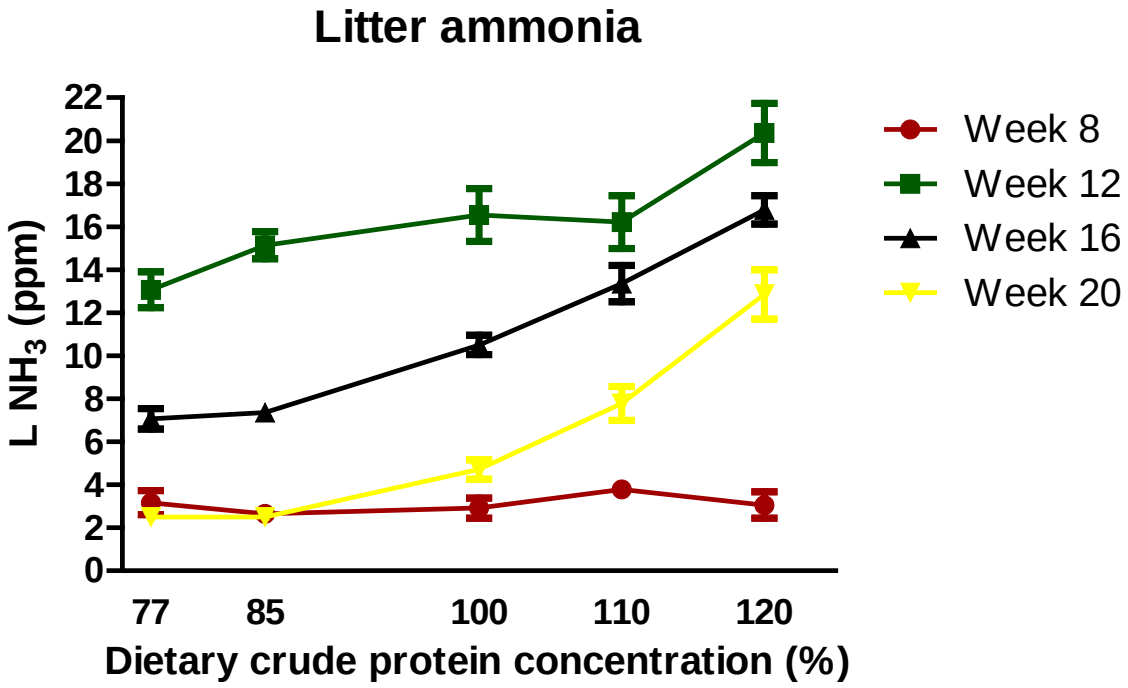


Figure 36: The effect of dietary CP concentration and growth phases on the trend of litter ammonia (L NH_3) in 20 week old turkeys (SEM bars correspond to each data point).

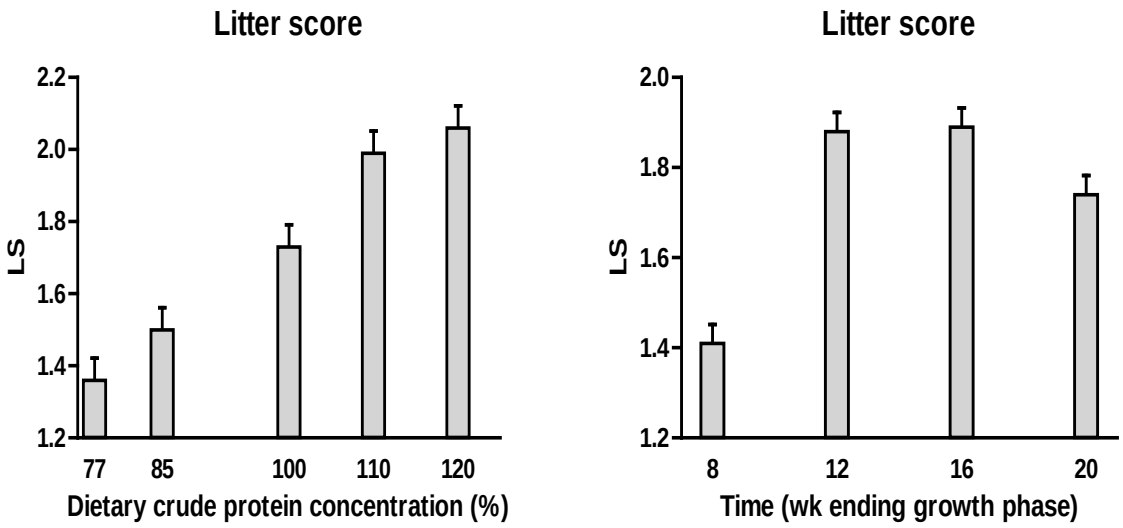


Figure 37: The effect of dietary CP concentration and time (growth phases) on the litter score (LS) in 20 week old turkeys (error bars represents pooled SEM).

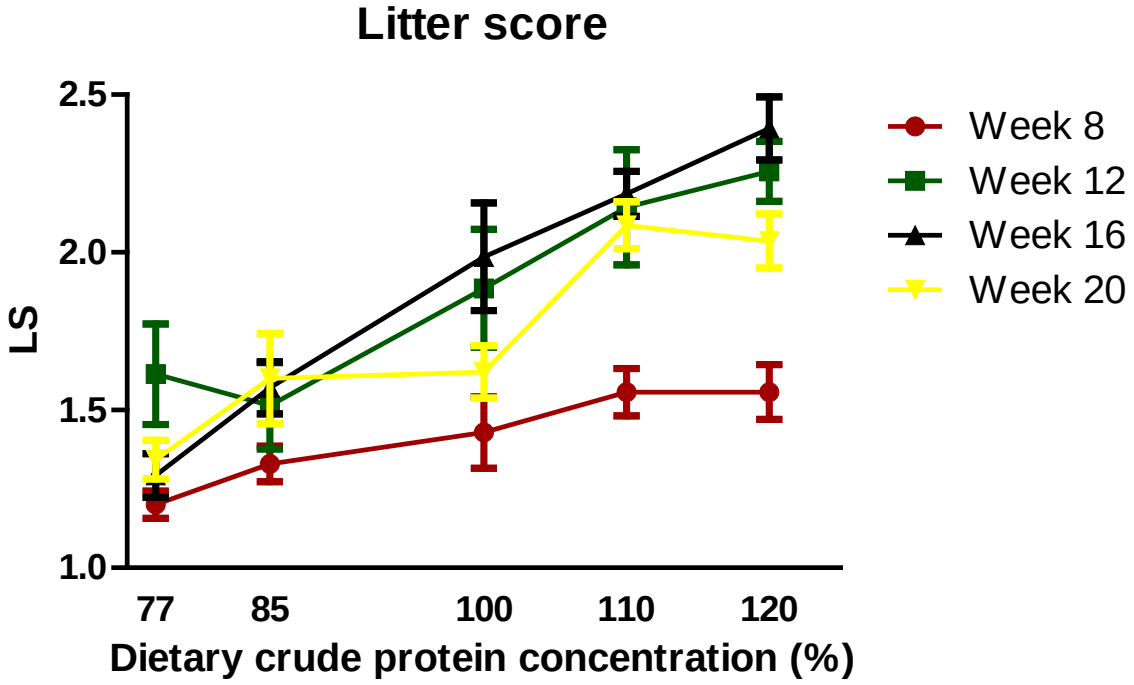


Figure 38: The effect of dietary CP concentration and growth phases on the trend of litter score (LS) in 20 week old turkeys (SEM bars correspond to each data point).

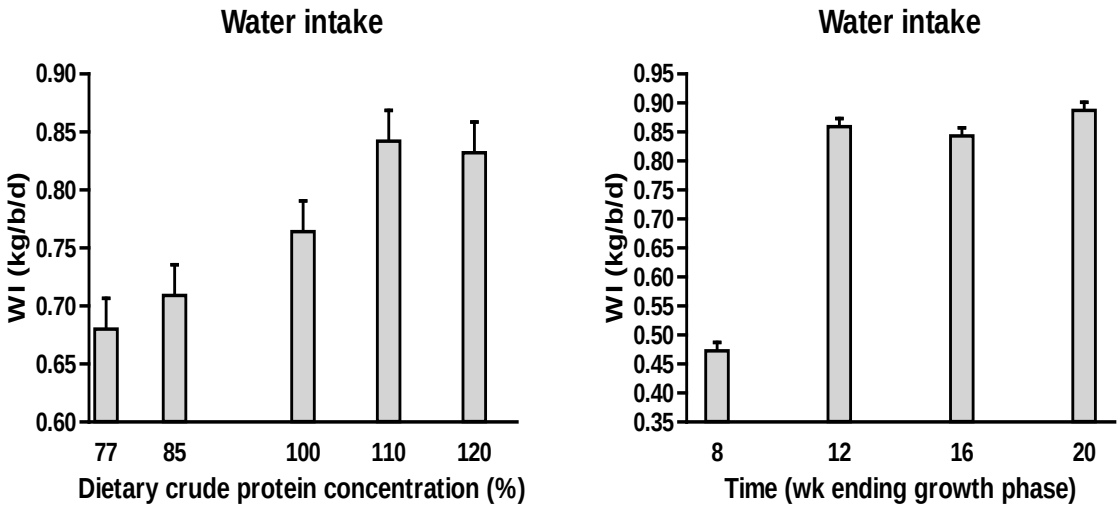


Figure 39: The effect of dietary CP concentration and time (growth phases) on the water intake (WI) in 20 week old turkeys (error bars represents pooled SEM).

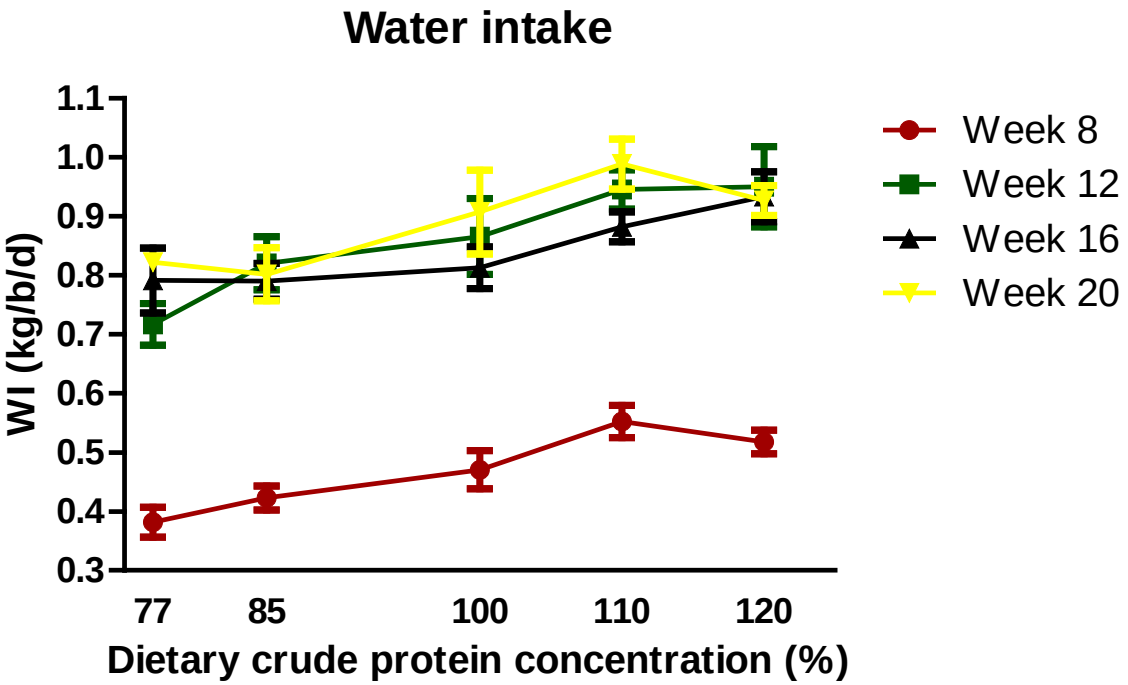


Figure 40: The effect of dietary CP concentration and growth phases on the trend of water intake (WI) in 20 week old turkeys (SEM bars correspond to each data point).

4.4 **Discussion**

4.4.1 Water intake measurements and Litter quality associated parameters

In the present study amino acid digestibility and CP digestibility coefficients showed that an increase in amino acid digestibility (disappearance from digesta) was concomitant with enhanced amino acid retention and therefore resulted in reduction in CP digestibility. Therefore an excess of dietary protein content resulted in a reduction in crude protein digestibility, and a possible increase in heat production that could have resulted in an increase in the water consumption. This may have led to an increase in moisture content of the litter. Similar findings were reported by Alleman & Leclercq (1997) and Zarate *et al*. (2003). A positive correlation between dietary crude protein and litter moisture content can be explained by Swennen *et al*. (2005). This study shows that birds fed on diets with a relatively high protein concentration can increase amino acid oxidation rate which resulted in excessive heat production and excretory nitrogen. This in turn increases water intake and water excretion by the bird, therefore, increasing moisture in the excreta (Tasaki & Okumura, 1964; Alleman & Leclercq, 1997). Relatively higher litter moisture and NH_3 concentration in 110 and 120CP fed turkeys in the present study suggested that a lower CP digestibility and high uric acid excretion by these birds were the contributing factors for higher litter moisture and NH_3 concentration. Some authors reported positive correlation of litter pH and moisture content with higher NH_3 emission from poultry litter (Carr *et al.*, 1990; Ferguson *et al.*, 1998). Therefore, a reduction in dietary protein content in the present study resulted in a lower nitrogen excretion by the birds and a lower NH_3 emission from the litter as supported by: (Blair *et al.*, 1999; Bregendahl *et al.*, 2002; Rezaei *et al.*, 2004). Uric acid is the end product of protein degradation in avian species and a substrate for litter NH_3 (Singer, 2003). Therefore, by reducing the available substrate (uric acid), less NH_3 will be formed and volatilized. Lower uric acid excretion in the present study was a confirmation of previous findings that feeding lower protein concentration resulted in a decrease in uric acid concentration in the blood (Rosebrough *et al.*, 1996; Collin *et al.*, 2003). Therefore, lower litter nitrogen or NH_3 content from birds fed lower protein diets in the present study was a direct consequence of the lower uric acid excretion by the birds. Even though factors such as NH_3 volatilization rate can be affected by litter moisture, litter pH, litter microbial load, or a combination of factors, however, no difference in litter temperature in the present findings indicated that there was no relationship of litter temperature with NH_3 release. In conclusion the results of present study that lower nitrogen excretion by the birds was a result of lower dietary protein were in line with previous findings (Jacob *et al.*, 1994; Elwinger & Svensson, 1996; Moran & Stilborn, 1996; Ferguson *et al.*, 1998; Hussein *et al.*, 2001; Bregendahl *et al.*, 2002; Rezaei *et al.*, 2004; Si *et al.*, 2004; Namroud *et al.*, 2008).

Dietary CP utilisation and faecal nitrogen excretion is less when we have an amino acid balanced diet (D'Mello, 1993; D'Mello, 1994; Moran & Stilborn, 1996). According to some reports (Holsheimer & Janssen, 1991; Ferguson *et al.*, 1998) an amino acid balanced lower dietary protein can ensure litter quality in poultry without any negative effects on performance. However, Han *et al*. (1992) highlighted that achieving optimum amino acid concentration using only the natural ingredients is not possible. Therefore, in the present study we adopted the approach of proportional decrease in amino acids along with protein content without disturbing the balance between them and to avoid any possible deficiency of non essential amino acids. We believed that attaining amino acid levels in diets with lower protein concentration through addition from synthetic source might end up creating imbalance between essential and non essential amino acids. This situation highly likely to lead to higher excreta moisture content since metabolic stress and excessive nitrogen excretion associated with excess and imbalanced dietary amino acid contents (Waldroup *et al.*, 1976), it is important to note that an amino acid imbalanced diet may also results in higher heat increment (Hurwitz *et al.*, 1980; Macleod, 1990; Brake *et al.*, 1994). Imbalance and antagonism amongst amino acids can have adverse affects on feed intake, body weight and feed efficiency (Sugahara *et al.*, 1969; Allen & Baker, 1972; Tews *et al.*, 1980; Davis & Austic, 1982a; Davis & Austic, 1982b; Cieslak & Benevenga, 1984; Cieslak & Benevenga, 1986), and even enzyme activities involved in the normal utilisation of amino acids (Wang *et al.*, 1973). According to Austic (1985) heat increment can be reduced by improving the amino acids balance, and by reducing dietary concentration above the requirement as a result it will also help reduction in nitrogen excretion.

Nagaraj *et al*. (2007b) found that protein source significantly affected the incidence and severity of FPD (all vegetable > vegetable + animal). The increased level of K^+ in the diet is related to the inclusion of higher levels of plant protein-rich ingredients especially soybean meal. Soybean meal is invariably the main source of vegetable protein in turkey diets however its is known to contain a relatively high amount of potassium (> 20 g/kg DM), which is an electrolyte known to increase water intake (James & Wheeler, 1949; Pesti *et al.*, 1999; Eichner *et al*., 2007) and therefore contribute to FPD when fed at high levels (Jensen *et al*., 1970; Nagaraj *et al*., 2007b). Indeed Vieira & Lima, (2005) have reported that chickens fed high vegetable protein diets have a higher water intake, possibly because of an ionic imbalance as well as other factors such as the increased non-starch polysaccharide content. Achieving higher protein concentration in the present study through higher inclusion of soybean meal, therefore, resulted in higher dietary potassium levels and was responsible for higher water intake and excretion rather than protein only. To address this question that whether water intake and excretion in turkeys is driven by protein or K^+ concentration or as a result of their interaction there is a need to

further investigate to strip out the effect of both on water intake and excretion in turkey production.

4.4.2 Leg health parameters

The high protein diets in the present study lead to a wet capped litter and severe hock burns (HB) and high litter moisture in combination with higher litter nitrogen content would have resulted in the condition favours the skin damage (Bray & Lynn, 1986). Since, higher litter moisture content might increase the rate of irritants released from the litter and sticky litter probably brings these irritants in permanent contact with the skin (Wang *et al.*, 1998). As there was an increase in the dietary CP concentration, so did litter NH_3 concentration causing irritation to the skin. However, another possibility is that low dietary protein would have resulted in an increase in the lipogenesis and, therefore, could have resulted in an increased resistance of skin against physical and chemical damage. This can be explained as higher protein intake causing a drop in plasma biotin level (Clark *et al.*, 2002) therefore, disrupting the biotin dependent lipogenic pathway involving acetyl-CoA carboxylase which then results in abnormal skin lipid composition and poor skin integrity. Poor skin integrity results in weak resistance against sticky faeces and micro organisms (Whitehead & Bannister, 1981; Clark *et al.*, 2002; Nagaraj *et al.*, 2007a; Nagaraj *et al.*, 2007b). Therefore, the high-protein diets could have been responsible for classical biotin deficiency signs leading to higher prevalence of incidences of skin damage (Bannister *et al.*, 1983). Since HB started earlier then footpad damage therefore, there could have been a shift in bird's behaviour, standing rather than sitting, due to pain in hock region. Therefore, litter NH_3 and pH and along with a change in litter contact time due to a probable shift in the behaviour might have been the reason of change in HB incidences.

4.4.3 Growth performance, dietary nutrient intake and utilisation

The positive relationship between the high levels of dietary protein, high feed intakes and body weights, and poor litter quality when compared to the birds fed relatively low concentrations of dietary protein agrees with previous reports where chickens were used (Ferguson *et al.*, 1998; Nagaraj *et al.*, 2007b). As indicated by D'Mello (1994), amino acid (AA) responses are better predicted by the absolute daily intake rather than by the dietary AA level. Therefore, in the present study birds fed the highest level of protein had the higher intake of amino acids and other nutrients and, therefore, higher weight gain. Since birds eat to meet energy requirement, so physical capacity and energy content can affect feed intake (Morris, 1968; Golian & Maurice, 1992; Leeson *et al.*, 1993). The increase in feed intake in groups fed diets containing higher protein concentrations indicates that AME concentration was not enough for the extra metabolic requirement for protein as

indicated by Musharaf & Latshaw (1999) that energy utilisation is poor from protein metabolism. Also noted by Emmans (1994) that protein synthesis require relatively large amount of energy i.e. 36J/g for protein as compared to 4 J/g for fat retention, so birds fed on diets with higher protein concentration increased their feed intake to compensate for any requirement of energy.

Growth may have been limited in birds fed diets containing the lower concentrations of dietary protein due to the inadequate amino acid supply, the birds being unable to increase their feed intake (due to similar AME across diets) to supply the amino acids required. Another possible reason can be that shift in ingredient composition might have affected palatability of the diets affecting feed intake as indicated by Wijtten *et al*. (2004). Therefore, the improved crude protein digestibility (CPD), dry matter digestibility (DMD) and organic matter digestibility (OMD) in the present study could not compensate for lower feed and amino acid intake in birds fed lower protein diets, hence had relatively lower body weight as compared to the one fed high protein diets.

There was no difference in protein efficiency ratios recorded across the treatments. However, energy efficiency ratio (different from efficiency of energy retention reported in literature where carcass composition rather than body weight was used to calculate EER) of birds fed with diets containing higher protein concentration was slightly better than those fed lower protein diets. It is important to note that difference in protein utilisation and energy utilisation at different dietary protein concentration reported in literature are focused mainly on the basis of carcass composition. As literature emphasised, the efficiency of protein utilisation depends on dietary protein concentration, but energy utilisation is dependent on energy to protein ratio therefore affecting carcass composition differently (Jackson *et al.*, 1982). Another possibility is that fat content of the carcass increases due to lower dietary crude protein this might have resulted in efficient energy utilisation when carcass composition was evaluated for different dietary regimes (Jackson *et al.*, 1982; Cheng *et al.*, 1997).

Mineral digestibility values in poultry can be affected by a number of factors. These can be cage material (plastic vs. metal), feather pecking, cannibalism, litter picking, interaction of minerals at site of absorption and excretion, and perhaps the most important of all, dissolved minerals in drinking water (Church, 1991). Although it was not the aim of this study to control these factors, a likely cause of overall lower mineral digestibility values in the present study can be their influence. Quadratic response of mineral digestibility to dietary protein concentration is possibly due to a similar response of feed intake to dietary protein concentration. Variation in the proportion of urates (uric acid bound with cations) in

the bird excreta (as a result of variation in dietary protein concentration) is another possible explanation (Roxburgh & Pinshow, 2002).

4.5 **Conclusion**

The data suggest that a reduction in dietary protein concentrations (with ideal amino acid ratio) with a constant metabolizable energy content (providing varying ME:CP) can:

- reduce water intake and excretion and therefore the litter moisture and NH_3 content
- improve overall litter quality
- reduce incidences of FPD and HB
- there was no negative effect of dietary CP reduction on PER and AME

The improvement in litter quality and a reduction in incidences of FPD may also be achieved by an increase in the dietary nitrogen digestibility and retention.

Chapter 5

The effect of dietary crude protein and potassium on water intake and excretion by turkeys

5 Aim

The aim of this experiment was to determine the interactions between protein and potassium in the context of soybean meal.

- water intake and excretion
- growth performance
- nutrient digestibility

5.1 Background

Since the EU-wide ban on the use of animal product for feeding farm animals came into force soybean meal (SBM) has become the main source of protein in poultry diet and it was SBM that was used in the preceding study where it was shown that water intake and excretion responded to dietary crude protein concentration. However as SBM contains significant amount of potassium (Eichner *et al*., 2007; Youssef *et al*., 2011), the concentration of this mineral increases as the protein concentration increases. The interpretation that water intake is responding solely to the protein concentration is therefore potentially flawed when using SBM as protein concentration is confounded by potassium concentration. So this study was designed to investigate possible interactions between protein and potassium and their influence on water intake and excretion, and, therefore, to improve our understanding of the relative importance of protein and potassium in water intake and excretion by growing turkeys.

5.2 Material and methods

The house was prepared as explained in Chapter 2 (Section 2.2.1). The dry matter, crude protein, mineral concentration, excreta moisture output and excreta moisture content, moisture output ratio weight gain and moisture output as % of water intake were determined as explained in Chapter 2 Sections 2.2.11, 2.2.12, and Chapter 3 Section 3.2.13.2. The calculations to obtain values of water intake, water to feed ratio, feed conversion efficiency and dry matter digestibility are given in Chapter 2 (Sections, 2.2.10, 2.2.8 and 2.2.9).

5.3 **Feed preparation**

In the pre-study period, from 0 to 7 days of age, the birds were fed a standard mash starter turkey feed, with the only exception of pellet binder replaced by wheat (Table 7, Chapter 3). The starter diet consisted of feed ingredients such as wheat, soybean meal, and fish meal and had a crude protein content of 274 g/kg and metabolisable energy (ME) of 12.5 MJ/kg.

Six wheat-soy-based experimental diets were offered to turkeys from 7 to 21 days of age (Table 48). Three basal diets were designed to contain 208, 274 and 330 g/kg dietary CP, that represents 77, 100 and 120% respectively, of the dietary protein recommended by the breeder (Aviagen Turkeys Ltd., UK) (Table 48). The total tract digestible amino acid values for different feed stuff determined by studies on caeca-ligated turkeys were used for feed formulation (Firman, 1992; Firman & Remus, 1993). The remaining values used were taken mainly from NRC (1994) recommendations. The digestible amino acid ratios were kept the same to maintain an ideal protein (IP) ratio in all diets (Table 8, Chapter 3), the amino acid ratios being adopted from breed recommendations and Firman & Boling (1998). The diet with 100% CP (IP) concentration (T2) was designed to be adequate in all nutrients recommended by the breeder (Aviagen Turkeys Ltd., UK) for 0-4 weeks of age. The diets T1 and T3 contained 77 and 120% concentration of crude protein (IP) respectively as compared to control diet T2, while maintaining the concentration of the rest of the nutrients. The three basal diets were then split in to two equal parts and one part of the respective basal diet was supplemented with potassium carbonate (K_2CO_3) (Sigma-Aldrich, Inc., St. Louis, MO 63103, USA) at 16.3, 11.81 and 5.9 g/kg diet, creating diets T4, T5 and T6 containing 208, 274 and 330g/kg dietary protein and all with 16.6 g/kg K^+ concentration respectively (Table 48). The potassium carbonate was added to the diets in powder form and all diets were fed as a mash. This gave two groups on the basis of K^+ concentration i.e. one group of feeds, including diets T1, T2 and T3, that had naturally occurring concentrations of K^+ (K^+_0), and another group of feeds, including diets T4, T5 and T6, that had a standardised K^+ concentration of 16.6 g/kg (K^+_T). Each diet was fed to five cage replicates (randomised complete block design). The K^+ concentration in the diets was formulated according to average values present within the various feed ingredients and according to the concentration of K_2CO_3 added (Table 48). To verify the actual amounts present samples representing each of the basal diets were analysed (Table 49).

Table 48: Ingredient and nutrient composition of experimental diets with different protein concentration and required K^+ used for turkeys for growth phase from 0-4 weeks of age.

Ingredients	Crude protein concentration (% of the commercial recommendations)					
	77 -T1	100 –T2	120 -T3	77 –T4	100 –T5	120 –T6
	g/kg					
Fish meal - (72%-CP)	30	30	40	30	30	40
Soybean meal - (48%-CP)	140	275	395	140	275	395
Wheat	748.3	585	452.5	732	573.2	446.6
Soy oil	10	17.4	27.6	10	17.4	27.6
Lysine HCl	2	1.9	1.5	2	1.9	1.5
DL Methionine	2	2.8	3.8	2	2.8	3.8
L-Threonine	2.1	3.9	5.5	2.1	3.9	5.5
Salt	2.2	2.2	0	2.2	2.2	0
Limestone	6.8	7	0	6.8	7	0
Corn gluten meal - (60%-CP)	0	20	20	0	20	20
Dicalcium Phosphate	23.3	21.5	1.3	23.3	21.5	1.3
Casein	30	30	30	30	30	30
Deflourinated Phosphate	0	0	19.5	0	0	19.5
Vit./min. premix[1]	2.8	2.8	2.8	2.8	2.8	2.8
K_2CO_3	0	0	0	16.30	11.81	5.90
Coccidiostat	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient analysis						
ME, MJ/kg[2]	12.3	12.15	12.15	12.3	12.15	12.15
CP (g/kg)	202.7	263.1	313.5	202.7	263.1	313.5
Crude fibre (g/kg)	22.8	29	29.8	22.8	29	29.8
Ca (g/kg)	10	10	10	10	10	10
Available Phosphorus (g/kg)	5	5	5	5	5	5
Na (g/kg)	1.6	1.6	1.6	1.6	1.6	1.6
Cl (g/kg)	2.3	2.3	2.3	2.3	2.3	2.3
K (g/kg)	6.3	8.4	10.3	16.6	16.6	16.6
Indispensable amino acids						
Arginine (g/kg)[3]	8.7	12.2	15.4	8.7	12.2	15.4
Cystine (g/kg)[3]	3.2	4.2	5	3.2	4.2	5
Isoleucine (g/kg)[3]	7.4	9.6	11.3	7.4	9.6	11.3
Lysine (g/kg)[3]	10.2	13.1	15.8	10.2	13.1	15.8
Methionine (g/kg)[3]	3.9	5.1	6.1	3.9	5.1	6.1
Phenylalanine (g/kg)[3]	7.9	10.5	12.4	7.9	10.5	12.4
Threonine (g/kg)[3]	6.2	8.1	9.7	6.2	8.1	9.7
Tryptophan (g/kg)[3]	2.3	3.1	3.8	2.3	3.1	3.8
Valine (g/kg)[3]	8.2	10.4	12.3	8.2	10.4	12.3
Dispensable						
Tyrosine (g/kg)[3]	7.1	9.4	11.1	7.1	9.4	11.1

[1]The vitamin and mineral premix (Target Feed Ltd) contained vitamins and trace elements to meet the requirements specified by the breeder. The premix provided (units kg^{-1} diets): Vit A 16,000 iu; Vit D_3 3,000 iu; Vit E 75 iu; Vit B_1 3 mg; Vit B_2 10 mg; Vit B_6 3 mg; Vit B_{12} 15 µg; Vit K_3 5 mg; Nicotinic acid 60 mg; Pantothenic acid 14.5 mg; Folic acid 1.5 mg; Biotin 275 µg; Choline chloride 250 mg; Iron 20 mg; Copper 10 mg; Manganese 100 mg; Cobalt 1 mg; Zinc 82 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.
[2]The ME value of the diet was calculated using the ME values of the dietary ingredients (NRC, 1994).
[3]Concentration of amino acid on digestible basis.

Table 49: Analysed nutrient composition of experimental diets.

Determined values	Crude protein concentration (% of the commercial recommendations)		
	77 -T1	100 –T2	120 -T3
Dry matter (g/kg)	856.6	858.8	856.6
Crude protein (g/kg)	178.3	235.4	282.8
Ash (g/kg)	55.2	64.0	66.5
K (g/kg)	6.3	8.4	11.4
Na (g/kg)	1.4	1.4	1.4
Ca (g/kg)	12.6	12.9	12.5
Total Phosphorus (g/kg)	10.0	10.2	10.1
Mg (g/kg)	1.4	1.5	1.9
Zn (mg/kg)	81.2	97.1	99.1

5.3.1 Animal husbandry

Sixty-five day old male turkeys (BUT 10) were weighed to get the initial weight and placed in a controlled environment house. For the first 7 days birds were placed on the floor in a pen containing 10 cm thick bedding material of wood shaving. Birds were offered a standard turkey starter mash diet for the first 7 days and had *ad libitum* access to feed and water.

On day 7, sixty turkeys were weighed, stratified on body weight and divided in to 5 groups of 12 each (from heaviest to lightest) and randomly allocated to the 5 spatial blocks. Within each group birds were ranked by weight and placed in 2 subgroups (heavy and light) with 6 birds in each. Each of these 6 birds in a subgroup was randomly allocated to 6 cages within the block. The process of randomization was repeated for the other subgroup of 6 birds in that particular block thus assigning both heavy and light subgroups randomly in each block. This practice resulted in maximal variation between blocks and minimum variation between replicates within blocks and so resulted in an increased power to detect treatment effects. The birds were reared in metabolism cages (two birds in a cage) providing 0.35 x 0.35 m floor area for 14 days, between 7 and 21 days of age, with each diet replicated five times in a randomised complete block design. Feed and water were offered *ad libitum* throughout the study, and water intake (WI) was determined daily. To determine moisture content in excreta, samples were collected daily from the trays located under each cage. Dry matter digestibility coefficient and moisture output (MO) were determined by total collection for the last 48h of the study. Excreta samples were collected at 4 hourly intervals and each collection was weighed and dried in an oven. The methods for DM and MO determination have been described in Chapter 2 Sections, 2.2.11 and 2.2.12.

5.3.2 Statistical procedure

Five replicates per treatment were used for the experiment with a total of sixty turkeys. A randomised complete block analysis of variance with a 2 x 3 factorial structure was used to compare the main treatment factors (dietary K^+ origin x dietary CP content). An orthogonal partitioning of dietary CP contents was used to quantitatively compare the regression effects. In all instances, differences were reported as significant at $P<0.05$. Genstat software, release 11 (IACR Rothamstead, Harpenden, Hertfordshire) was used to perform factorial ANOVA for the comparison of different treatments for DM output, WI, FI, WG, FCE. Least significant difference (LSD) was used to determine which means amongst the set of treatments means differ from the rest. Differences were reported as significant at $P<0.05$ and trends were noted when the P value was near to 0.1.

Multiple linear regression analysis was used to assess the relationship between variables of turkey's water intake and moisture output, and the crude protein or potassium intake. A step-wise regression technique selected the terms to add as explanatory variables into a linear model. The two variables describing water intake and moisture output were used separately as the dependent variables. The daily intakes of crude protein and potassium were offered as terms in the multiple linear regressions.

5.4 **Results**

Analysed chemical composition of the basal diets is presented in Table 49. The analysed values for the concentration of crude protein (CP) and potassium (K^+) content were lower than the calculated values whereas the analysed Ca concentration was higher than the calculated values in Table 48.

5.4.1 Water intake and excretion

Overall, birds fed lower dietary CP (diet T1) had approximately 32 and 38% lower ($P<0.001$) water intake, about 53 and 121% lower ($P<0.001$) moisture output, almost 14 and 38% lower ($P<0.001$) MO/WI% and about 6% higher and 22% lower ($P<0.01$) MO:WG, when compared to birds fed T2 and T3 diets, respectively. Each gram of dietary CP increased by 5.8 g the daily water intake per bird. Turkey fed diet T2 excreted 44.6% less ($P<0.001$) moisture than those fed diet T3. There was a significant ($P<0.001$) linear response of water intake and moisture output to dietary CP concentration (Figure 41 and Figure 42). The daily moisture output was increased by 3.6 g per bird with each gram increase of dietary CP. However, bird quantitatively consumes more CP as compared to

K^+ intake, as dietary CP concentration is far higher than K^+ concentration. As indicated by r^2 values (Table 52) the effect of CP was more significant than that of K^+.

There was an interaction between source of K^+ and dietary CP concentration for water to feed ratio ($P<0.001$) and excreta moisture ($P<0.01$) (Table 51). The interaction was due to different response of water to feed ratio and excreta moisture to dietary CP concentration in diets containing standardised potassium content (K^+_T) compared to diets containing naturally occurring potassium (K^+_0). For example, the response of WF ratio to CP seems to follow a linear pattern for diets T1, T2 and T3, although the shape of response for diets T4, T5 and T6 was quadratic. The excreta moisture content response to dietary CP followed a quadratic pattern for diets T1, T2 and T3, but the response of diets T4, T5 and T6, was relatively minor, and not parallel to those observed with the first three diets.

Birds fed naturally occurring potassium content (K^+_0) had about a 10 and 18% lower ($P<0.01$ and $P<0.05$) moisture output, moisture output ratio weight gain (MO:WG), respectively and tended ($P=0.06$) to have lower water intake (WI) when compared to those fed K^+_T. However, there was no significant difference ($P>0.05$) recorded in moisture output as % of WI (MO/WI%) for dietary potassium concentration source. Each gram of K^+ intake was responsible for 94.6 g increase in water intake, and 48.5 g increase in moisture output (Table 52).

The results of the regression analysis are presented on Table 52. According to regression analysis per gram of protein intake can increase average daily water intake (ADWI) by 5.8g (Table 52) and for average daily moisture output (ADMO) 1g of CP intake can increase MO by 3.6g. Regression analysis showed that per gram of K^+ intake ADWI increased by 94.6g (Table 52) and for ADMO 1g of K^+ intake could increase MO by 48.5g. However, birds quantitatively consume more CP as compared to K^+ intake, as dietary CP concentration is far higher than the K^+ concentration. As indicated by r^2 values (Table 52) the effect of CP was more significant than that of K^+.

5.4.2 Turkey's performance and dry matter digestibility

Overall body weight was lower than the breed standards at 21 days of age, 495 g vs 630 g expected. The body weight (BW) and dry matter digestibility (DMD) of the birds fed diets contains naturally occurring K^+ (K^+_0) were significantly ($P<0.05$) higher by 8 and 3%, respectively, when compared to the same parameters of the birds fed K^+_T diets (Table 50). There were no differences ($P>0.05$) between the feed conversion efficiency (FCE), feed intake (FI) and weight gain (WG) of the birds fed K^+_0 or K^+_T diets.

Overall, birds fed the low protein diet (T1) had lower growth performance when compared to birds fed diets relatively high in protein (diets T2 and T3). The body weight of birds fed diet T1 was 32 and 38% lower ($P<0.001$) than those of birds fed diets T2 and T3, respectively, and the response of body weight to CP concentration was a linear function ($P<0.001$) with increasing CP concentration (Table 50). Birds fed diets T1 had a lower ($P<0.01$) feed intake than birds fed diets T2 and T3 (Table 50) about 17.5 and 17.7% lower respectively. The differences in feed intake were best described as a linear function ($P<0.01$) of the CP concentration. The weight gain of the turkeys fed diet T1 was 55 and 64% lower ($P<0.01$) than the gain of turkeys fed diets T2 and T3, respectively. There was a significant linear response ($P<0.001$) of the weight gain to dietary CP concentration. The FCE of the birds fed diet T1 was 33 and 40% lower ($P<0.001$) than the FCE of turkeys fed diets T2 and T3, respectively. However, birds fed T3 had approximately 6% higher ($P<0.001$) FCE than those fed diet T2 (Table 50). Similar to the rest of the growth parameters determined in the study, the response of the FCE to CP concentration was a linear function ($P<0.001$). Interestingly, dietary DM digestibility coefficients of birds fed diet T1 were about 5.5 and 8% higher ($P<0.01$) than those of birds fed diets T2 and T3, respectively, (Table 50), and the response of dry matter digestibility to CP concentration was a linear function ($P<0.001$). No protein by potassium source interactions was detected ($P>0.05$) with regard to BW, FI, WG, FCE and DMD.

Table 50: Effect of dietary crude protein and potassium on body weight (BW), feed intake (FI), weight gain (WG), feed conversion efficiency (FCE) and dry matter digestibility (DMD) in turkeys for 7-21 days of age.

Treatment factors	BW (g)	FI (g/b/d)	WG (g/b/d)	FCE	DMD (g/kg)*
K^+ concentration					
Naturally occurring (K^+_0)	514.1	55.6	38.5	0.694	703.2
Standardized (K^+_T)	476.0	53.7	35.8	0.671	686.6
SEM	12.23	1.54	1.20	0.0283	4.79
Dietary crude protein (CP, g/kg)					
208	401.2[a]	48.9[a]	26.6[a]	0.551[a]	728.3[b]
274	530.2[b]	57.5[b]	41.2[b]	0.728[b]	688.6[a]
330	553.8[b]	57.5[b]	43.6[b]	0.769[b]	667.8[a]
SEM	14.98	1.89	1.47	0.0347	5.86
K^+ concentration x CP concentration (g/kg)					
K^+_0 + 208	439.4	51.6	29.7	0.576	738.6
K^+_0 + 274	538.7	58.5	42.3	0.739	692.4
K^+_0 + 330	564.3	56.7	43.5	0.768	678.6
K^+_T+ 208	363.1	46.2	23.4	0.525	717.9
K^+_T+ 274	521.7	56.4	40.1	0.718	684.7
K^+_T+ 330	543.2	58.3	43.8	0.770	657.1
SEM	21.18	2.67	2.08	0.0490	8.29
Probabilities of statistical differences					
K^+ concentration	<0.05	NS	NS	NS	<0.05
Dietary crude protein (CP g/kg)	<0.001	<0.01	<0.001	<0.001	<0.001
Linear	<0.001	<0.01	<0.001	<0.001	<0.001
K^+ concentration x CP concentration	NS	NS	NS	NS	NS

*Dietary DMD was determined between 19 and 21 days of age; There is a statistical significant difference when P<0.05; SEM- Standard errors of means; means within a column with no common superscript differ significantly. There were 5 observations per treatment.

Table 51: Effect of dietary crude protein and potassium on water intake (WI), water to feed ratio (W:F), excreta moisture content, excreta moisture output (MO), moisture output as % of water intake (MO/WI%) and moisture output ratio weight gain (MO:WG) in turkeys for 7-21 days of age.

Treatment factors	WI (g/b/d)	W:F (g/g)	Excreta moisture content (g/kg)	MO (g/b/d)	MO/WI% (g/g x100)	MO:WG (g/g)
K^+ concentration						
Naturally occurring (K^+_0)	113.4	2.03	679.1	43.7	37.92	1.12
Standardized (K^+_T)	123.6	2.29	706.9	48.0	38.86	1.38
SEM	3.67	0.015	2.43	0.91	1.371	0.066
Dietary crude protein (CP, g/kg)						
208	96.0 [a]	1.97[a]	677.0[a]	29.0[a]	30.45 [a]	1.16 [a]
274	126.7 [b]	2.21[b]	699.7[b]	44.4[b]	35.35 [a]	1.09 [a]
330	132.8 [b]	2.31[c]	702.3[b]	64.2[c]	49.34 [b]	1.49 [b]
SEM	4.49	0.018	2.98	1.11	1.679	0.804
K^+ concentration x CP concentration (g/kg)						
K^+_0 + 208	91.8	1.78[a]	653.9[a]	26.2	28.59	0.89
K^+_0 + 274	121.2	2.07[b]	690.0[b]	43.1	35.73	1.03
K^+_0 + 330	127.3	2.25[d]	693.4[b]	61.8	49.43	1.45
K^+_T+ 208	100.2	2.16[c]	700.2[bc]	31.9	32.31	1.43
K^+_T+ 274	132.1	2.34[e]	709.3[c]	45.6	34.98	1.16
K^+_T+ 330	138.4	2.37[e]	711.2[c]	66.6	49.26	1.54
SEM	6.35	0.025	4.21	1.57	2.374	0.114
Probabilities of statistical differences						
K^+ concentration	P=0.06	<0.001	<0.001	<0.01	NS	<0.05
Dietary crude protein (CP g/kg)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05
K^+ concentration x CP concentration	NS	<0.001	<0.01	NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- Standard errors of means; means within a column with no common superscript differ significantly. There were 5 observations per treatment.

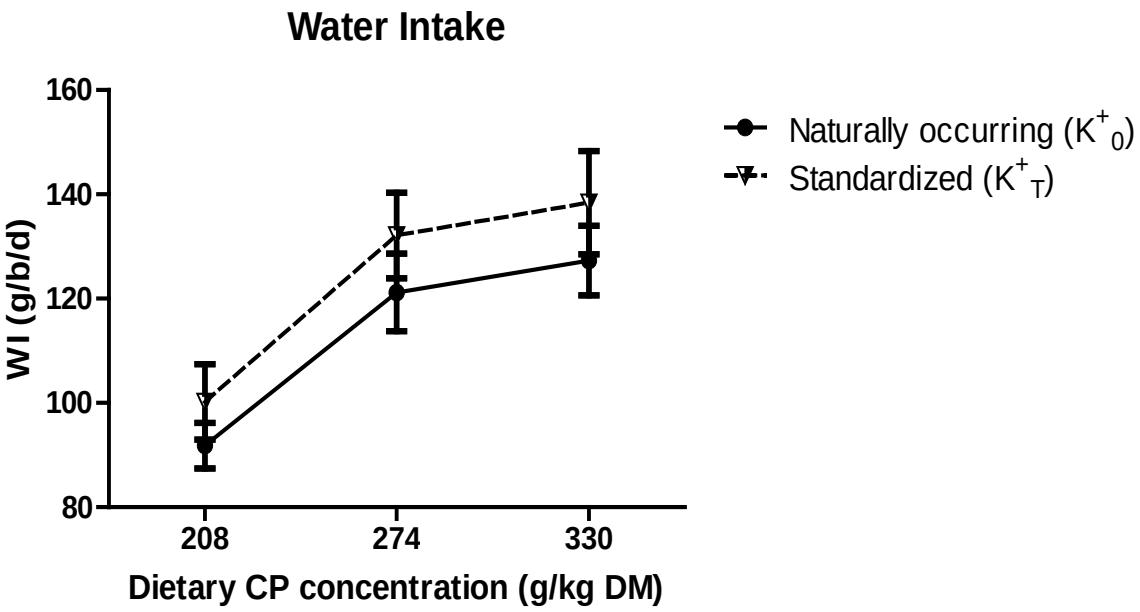


Figure 41: The effect of dietary CP (g/kg) and K^+ (g/kg) concentration on WI in turkey production.

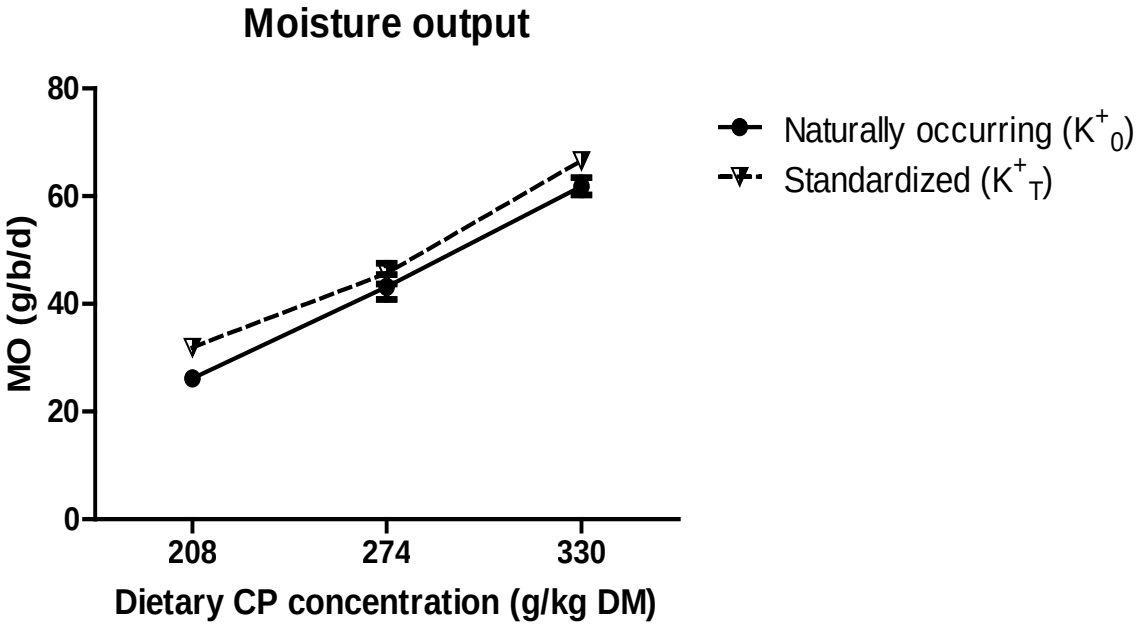


Figure 42: The effect of dietary CP (g/kg) and K^+ (g/kg) concentration on MO in turkey production.

Table 52: The relationship between average daily water intake (ADWI, g/b/d) and average daily moisture output (ADMO, g/b/d) of turkeys and their daily intake of various independent variables (g/b/d).

Dependent variate	Explanatory variates constant	Crude protein intake (g/b/d)	Potassium intake (g/b/d)	r^2	Residual standard deviation
ADWI	44.36 (±7.09)	5.77 (±0.532)		0.80	10.3***
ADWI	58.90 (±8.84)		94.6 (±13.4)	0.63	14.0***
ADWI	34.52 (±5.26)	4.20 (±0.467)	47.63 (±8.61)	0.90	7.15***
ADMO	-0.22 (±5.70)	3.59 (±0.427)		0.71	8.24***
ADMO	15.36 (±7.63)		48.5 (±11.6)	0.36	12.1***
ADMO	-2.94 (±5.97)	3.15 (±0.530)	13.17 (±9.78)	0.71	8.12***

Statistical significance of regression equation: ***P<0.001. Values in parenthesis represent the standard error. Whereas, ADWI, ADMO stands for the average daily water intake and average daily moisture output on g/bird/day basis.

5.5 **Discussion**

The analysed dietary concentration of CP and K^+ contents were slightly lower and of Ca were higher than the calculated values, which was probably due to differences between the composition of the actual ingredients that were used in the present study and the values provided by NRC (1994) for the same ingredients. The relatively low final body weight of the birds, when compared to breeder's standards, may be explained partly by the rearing conditions, i.e. cage with wire meshed floors, and also by the lower protein concentration as well as possible negative effect of higher dietary K^+ concentration.

Little information is available on the effects of relatively high dietary concentrations of K^+ on performance, water intake and excretion, as well as on nutrient utilisation in turkeys.

5.5.1 Water intake and excretion

To maintain the homeostatic environment in the body, birds obtaining more minerals, e.g. K^+, will require more water (Borges *et al.*, 2003b; Borges *et al.*, 2004). If concentration of the K^+ in the GIT of the bird exceeds the needed for a normal physiological functioning, the rest would be excreted, and will also require an increase in water excretion (Oviedo-Rondon *et al.*, 2001). All this would increase enormously the pressure on the kidneys of the birds, which may develop an acute renal failure that can adversely affect water re absorption through kidneys in turkeys (Reece *et al.*, 2000) and further increase the moisture output.

The positive linear (P<0.05) response of water intake to dietary CP indicates that the effect of CP was more significant than that of K^+ on water intake suggesting that protein

rather than potassium was the main driver of water intake in turkeys. However, non significance (P>0.05) difference between groups fed diets containing 274 and 330 g/kg of CP indicates that the magnitude of the difference between the lower dietary CP (i.e. 208 g/kg) and higher levels was not same in terms of the effects on water intake. Although in case of water to feed ratio there was a significant effect of potassium concentration or origin on this parameter but the highest values of water to feed ratio were still noted in groups of bird fed with diets containing higher CP concentration. Close agreement to present findings Larbier & Leclercq (1994) found that each g/kg increase in dietary crude protein (CP) intake increases water intake by 3g/kg. Higher heat increment (HI) associated with protein metabolism (Emmans, 1994), can increase water requirement of animal for the dissipation of this extra energy (Pfeiffer *et al*., 1995), and, therefore, can cause increase in moisture content of the excreta consequently increase litter moisture, pH and NH_3 (Ferguson *et al.*, 1998).

Youssef *et al*. (2011) reported that turkeys fed the high SBM diet were observed to have a markedly higher water intake than the others in following order (Soybean meal > K^+ > Oligosaccharides > control) and their excreta appeared visually wet or sticky, a feature of soybean and a cause of higher incidences of FPD (Jensen *et al*., 1970). Since in this study the dietary protein level was achieved by increasing inclusion levels of soybean it is quite possible that the response to water intake at the higher level of dietary protein may result from the combined effect of protein, naturally present K^+ as well as the oligosaccharide content of the soybean meal.

The results of this study also indicate that dietary K^+ may influence excreta moisture content and moisture output (MO), although the effect of dietary CP was more pronounced on these parameters. These findings are supported by the work of Namroud *et al*. (2008) who reported a higher excreta NH_3, pH and moisture content in broiler chickens when diets containing higher protein were compared with lower concentration even when all diets had similar mineral concentrations. Elwinger & Svensson (1996) working on broiler and Jirjis *et al*. (1997) on turkeys, also found that an increase in dietary protein content increase urinary volume and NH_3 emission. O'Dell *et al*. (1960) reported that the sources and level of dietary protein can influence the distribution of urinary nitrogen between uric acid and ammonia. Although the underlying mechanism of the correlation between water intake with ammonotely remains obscure (Aldea & Sabat, 2007), ammonia is osmotically active and toxic and, therefore, requires a significant amount of water to detoxify and excrete it (Mcnabb *et al.*, 1972; Wright, 1995) (see Figure 3 for details). This indicates that excreta moisture content and other litter quality parameters (e.g. NH_3 and pH) were closely associated with dietary protein concentration rather than minerals, as the magnitude of effect by K^+ origin was less as compared to

dietary protein concentration. Likewise, in the present study for potassium origin the values of water to feed ratio, excreta moisture content and MO were recorded as 13, 4 and 10% lower, respectively, for birds fed diets containing K^+_0 compared to birds fed diet contain K^+_T. Similar findings were reported by Shaw *et al*. (2006) working on pigs, who reported water intake was driven by CP concentration in the diet and not by the minerals concentration. They also suggested that minerals might have impact on water excretion through faeces rather than through urine as they did not find any difference in urine osmolality. The effect of dietary potassium concentration on excreta moisture content was highlighted by Koreleski *et al*. (2010) in a study on broiler as they reported lower excreta dry matter content when dietary concentration of potassium was high.

5.5.2 Turkey's performance and dry matter digestibility

In the present study, weight gains and feed intakes of turkeys fed standardised K^+ concentrations tended to be lower when compared to those of birds fed diets containing naturally occurring K^+ only. The trends observed is in agreement with the reports where turkeys fed diets containing over 12 g/kg dietary K^+ concentrations resulted in decreased weight gain compared to birds fed lower K^+ concentrations (Chavez & Kratzer, 1973; Smith *et al.*, 1973; Reece *et al.*, 2000). Reese *et al*. (2000) found that turkeys fed high K^+ concentrations had poor performance, e.g. inappetence, and grow, and begun to excrete faeces with high moisture content as a result of poor tolerance of turkeys to high dietary K^+ contents. The same authors (Reece *et al.*, 2000) also reported a significant increase in plasma K^+ when a diet containing higher K^+ was fed to turkeys. However, Scott & Austic (1978) reported a positive effect on weight gain of chickens when fed relatively high dosage of dietary potassium (18 g/kg) in high lysine concentration diets. Most of the studies indicating a better nutrient utilisation in broilers raised at higher ambient temperature were mainly due to higher water intake when supplemented with KCl (Dai *et al.*, 2009). However, the reported values for potassium concentration by Oliveira *et al*. (2005) and Smith & Teeter (1992) as well as that of Naseem *et al*. (2005) were far lower, in the range of 8-10 g/kg of diet, than used in the present experiment.

Higher dietary protein level resulted in higher body weight gain and therefore, feed intake however, there could also be some other reasons of higher feed intake. As explained by Musharaf & Latshaw (1999) protein metabolism require relatively more energy than carbohydrates and fats, which can affect energy demand of the bird. Since turkey is naturally a lean meat producing bird and, therefore, fixes more protein in muscles as compared to other poultry and also nutritionally contain higher dietary CP concentration. So to meet energy requirement in the present study, where all dietary treatments contained similar ME, birds were eating more feed at higher dietary CP content. This

higher feed intake could be due to a higher heat increment (HI) associated with protein metabolism (Emmans, 1994). Since osmotic properties of protein and amino acids force bird to maintain homeostasis within the body, and since osmoregulation is a physiological phenomenon that require a lot of energy, to meet the energy requirement birds had higher feed intake. Higher feed intake can result in to poor dry matter digestibility, there is also a possibility of poor dry matter digestibility when protein intake is higher as described by Tendoeschate *et al*. (1993). In the present study, we recorded a better dry matter digestibility when dietary protein concentration was lower. Similar type of findings was reported by Wolde *et al*. (2011) that optimum nutrient utilisation and retention was obtained at lower dietary CP concentration for Rhode Island Red chickens.

It was also observed that higher dietary protein levels produced significantly lower dry matter digestibility, but that potassium did not have the same effect. Turkeys fed diets containing 208 g/kg of CP had a higher dry matter digestibility compared to birds fed 274 and 330 g/kg dietary CP – about 6 and 8% respectively. Whereas for potassium origin the value of dry matter digestibility was recorded as 2% higher for birds fed diets containing K^+_0 compared to birds fed diet contain K^+_T. Similar type of findings were reported by Koreleski *et al*. (2010) that nitrogen retention decrease with an increase in mineral concentration in the diets of broiler chicken. Potassium standardization resulted in depressed FCE which might be a reflection of lower dry matter digestibility and, therefore, resulted in pronounced effect on excreta moisture content as compared to CP concentration in the diet.

5.6 **Conclusion**

It was observed that dietary protein levels associated with the inclusion of SBM had positive linear effect on excreta moisture content and a negative linear on dietary dry matter digestibility, although dietary potassium did not have the same effects at lower dietary protein level but it was not to the same extent at higher levels. On the basis of a significant linear effect of dietary CP and results from regression analysis showing that the effect of CP on water intake was more significant than that of K^+. It was concluded that for SBM the protein was the main determinant of water intake with potassium playing a secondary role.

This study was designed to assess the relevant importance of protein and potassium in SBM, the main protein source used in poultry diets. However the influence of other associated factors, such as NSPs, antinutritional factors (ANFs), cannot be totally excluded. To do this a synthetic or semi-synthetic diet could be formulated that incorporated the variable concentrations of CP and potassium.

6 General conclusions

The aim of this project was to improve understanding of the interaction between protein, energy and water intake.

The review of the literature showed that water intake is related to litter moisture which in turn is correlated with the incidence of footpad dermatitis (FPD) and hock burn (HB). Several factors influence water intake and excretion by birds, but the most important factors are the quantity of nutrient intake and their digestibility. The dietary factors interact in a complex manner to influence water excretion, but important dietary factors are the structure and chemical composition of carbohydrates, source and level of fats as well as the level of, and interaction between, minerals in the diet. Dietary protein is however probably the most important dietary factor, the source of the protein as well as the balance of its amino acids. In view of these interacting dietary factors, dietary manipulation promises to be an important way of improving litter quality and so reducing FPD in turkey.

The first study (reported in Chapter 2) showed that nutrient density dilution using sand resulted in improved organic matter and dry matter utilization in turkeys. The improvement in nutrient utilization resulted in lower excreta moisture content, hence drier litter. However, supplementation of diets with phytase resulted in higher moisture excretion by turkeys for the same body weight gain highlighting the need for dietary mineral reduction when diets are supplemented with phytase and reinforcing the importance of minerals when considering water intake.

The second study (reported in Chapter 3) assessed the influence on water intake of varying the concentrations of dietary energy and protein while maintaining a constant ratio between the two. This study demonstrated that increasing the concentration of apparent metabolisable energy (AME) and crude protein (CP) while keeping their ratio constant was important for reducing litter moisture content. Lower litter moisture content was observed in turkeys fed diet with a higher AME and CP concentration although it was notable that the litter NH_3 concentration was higher. Although there was no difference in FPD incidence between the dietary treatments, significantly higher incidences of HB in turkeys fed diets with higher AME and CP concentration was observed. This was thought to be a result of the interaction between litter quality parameters such as litter moisture and NH_3.

In the third study (reported in Chapter 4) the effect of higher energy to protein ratio on water intake was investigated. Since our second experiment suggested that there were interactions between aspects of litter quality such as NH_3 and moisture, the third experiment was designed to investigate this further. It was demonstrated that the ratio of

energy to protein, rather than the absolute levels of these in the diet is important in reducing litter moisture and the NH_3 content and therefore incidences of leg health problems. Lower litter moisture content, NH_3 and pH were observed in turkeys fed diets with a higher AME:CP. The birds on these diets also had lower incidences of FPD and HB damage.

The fourth and final study (reported in Chapter 5) was done to determine the effect of crude protein and potassium on water intake and excretion by turkeys. Having investigated the role of protein and energy in previous experiments, this study investigated possible interactions between protein and potassium and their influence on water intake and excretion. It was observed that higher dietary protein levels produced a lower dry matter digestibility and higher excreta moisture content, but that potassium did not have the same effect. It was concluded that it was likely (as associated factors such as NSPs, ANFs cannot be totally excluded) the dietary protein content associated with inclusion of soybean meal in the diet was primarily responsible for determining water intake and hence excretion.

Overall the studies have shown that dietary protein is responsible for the higher water intake and excretion that ultimately results in poor litter quality and associated leg health problems in turkeys. Excessive protein intake can be controlled by an increase in ratio of AME to CP, while maintaining the balance of amino acid ratios in diets.

6.1 **Discussion**

Researchers studying poultry nutrition have a range of tools at their disposal (e.g. precision feeding, modelling, digestible formulation, and concepts such as ideal protein). However, the focus of such research has tended to be the most common species and lines, the chicken or laying hen. As a consequence the ability of nutritionists to support the turkey industry has been limited by the lack of research data. This places the turkey at a notable disadvantage as inadequate or imbalanced diets can affect not only growth but other, sometimes welfare related, factors such as litter quality and hence skin lesions such as pododermatitis and hock burn. Therefore the objectives of this dissertation are: to provide information on the suitability of the current amino acid and dietary energy requirements in terms of their effects on water intake and hence excretion as well as the related impact on litter quality and consequently contact dermatitis in turkey production.

In terms of dietary constituents protein and minerals are two of the primary candidates that, may affect water intake and hence excretion (Murakami *et al*., 2000; Francesch & Brufau, 2004; Shaw *et al.*, 2006; Ziaei *et al*., 2007). In this study it was found that

increasing the concentration of dietary protein (as provided primarily by SBM) while maintaining a constant metabolizable energy content (ie varying the ME:CP) had a positive and linear effect on water intake, findings consistent with those of Elwinger & Svensson (1996), (Ziaei *et al*., 2007) and others. However incase of nutrient density (CP and AME (reported in Chapter 3) on the contrary resulted in lower water intake. The lowering of nutrient density resulted in increased feed intake as birds were trying to meet energy requirement, this increase in feed intake hence resulted in incraesd water intake. However SBM is known to contain a number of factors besides protein that may affect water intake, in particular potassium, hence raising concerns that interpretation of the data could be confounded by dietary potassium concentrations. However when the concentration of dietary potassium was manipulated (see Chapter 5) it was concluded that, for SBM anyway, the protein was the main determinant of water intake with potassium playing a secondary role. It is notable however that while, like potassium, high concentrations of sodium (Na) have been reported to result in increased water intake (Appleby *et al*., 1992; Tucker & Walker, 1992; Fuller *et al*., 2004); however, very high intakes can reduce water consumption, probably because of the accompanying anorexia (Reece *et al*., 2000). These findings are consistent with the results of the present study. So, when the potassium concentration was maintained at 16.6 g/kg of the diet (achieved by adding K_2CO_3) birds had a reduced feed intake, potentially confounding the data and contributing to the non-significant increase in water intake compared to non-supplemented groups. However the concentrations of potassium that occur naturally in SBM fall within the limits that would preclude a reduction in feed intake (Reece *et al*., 2000). Related to these findings though are results reported in Chapter 2, where water intake was increased possibly as a result of a mineral imbalance resulting from the use of supplementary phytase. Phytase has been implicated in increasing water intake in studies reported previously (Cowieson *et al*., 2004).

A recent investigation from Youssef *et al*. (2011) reported that the turkeys fed a high SBM diet were observed to have a markedly higher water intake than the others in following order Soybean meal > K^+> Oligosaccharides. The excreta appeared visually wet or sticky and so had the potential to increase the incidence of FPD (Jensen *et al*., 1970). Similarly in the present studies it was observed that higher dietary protein levels produced a lower dry matter digestibility and higher excreta moisture content but, with potassium having been accounted for, it was concluded that it was likely (as associated factors such as NSPs, ANFs cannot be totally excluded) that the dietary protein content associated with inclusion of soybean meal in the diet was primarily responsible for determining water intake and hence excretion. So while it is concluded that for SBM it is the protein that is the main driver of water intake that is not to say that under appropriate conditions other factors, such as minerals have a negligible or, in some instances, overriding effect.

Generally therefore an increase in dietary protein levels resulted in increased excreta/litter moisture content. However apparently contradictory results were obtained when comparing the results of the nutrient density studies reported in Chapters 2 and 3. In these studies nutrient density was adjusted by the use of wheat bran and sand. Wheat bran increases the fibre content of the diet, in particular the NDF fraction, leading to physico-chemical problems – increased digesta viscosity, water intake and moisture in the gut – which in turn reduce nutrient availability and increased moisture in the excreta (see Chapter 3). This in turn resulted in poor DMD and was believed to be main reason of higher than normal water excretion. On the contrary where sand was used to reduce nutrient density the DMD digestibility improved with lowering nutrient densities (possible grinding effect of sand) and therefore lower excreta moisture content (see Chapter 2). These studies highlight the importance of potentially confounding factors when undertaking studies on this topic. However it was concluded that water intake and excretion was not only affected by higher feed intake but also by DMD depending upon whether or not a grinding substance (e.g. sand) is present in the diet (Ziaei *et al*., 2007).

Since turkey excreta typically contain up to 85% water anything that increases excreta production (eg higher indigestible OM) will result in an increase in water intake and hence excretion of water into the environment. Undigested materials and excessive nutrients in large intestine can increase osmolarity of digesta (Etheridge *et al*., 1984) so, conversely, improving nutrient digestibility (as reported in Chapters 2, 3, 4 and 5) can reduce osmotic pressure in the GIT and body and hence has the potential to reduce moisture excretion. Higher water intake does not always means higher excretion. Providing water intake is in proportion to the increase in productive output (muscle mass) then any differences in water consumption in growing birds should not be a problem. However if the increase in water consumption is in response to the indigestible proportion of feed then it can lead to a wet litter problem.

Amino acid digestibility and CP digestibility coefficients some times vary inversely (Jirjis *et al*., 1997). So an increase in amino acid digestibility (disappearance from digesta) can fail to reflect an increase in amino acid retention, hence the reduction in the CP digestibility which is especially important when water utilization is in consideration. It was established through these studies (see Chapter 3 and 4) that a lower intake of feed in general and the nutrient in question in particular can increase bird's efficiency to utilize them more effectively, findings that are consistent with a number of previously reported studies (eg Marks & Pesti, 1984; Skinner *et al*.,1993; Weurding *et al*.,2001).

Increasing dietary protein levels (providing varying ME:CP) resulted in increased litter scores (reflecting the worse quality litter). This was however not true when the absolute

concentration of CP and AME (similar CP:AME) was compared. Under these circumstances (reported in Chapter 3) the lowest water intake, litter moisture and hence litter scores were recorded when birds were fed diet containing the highest amount of CP and AME.

Although some studies (Sorensen, 1992; Kjaer *et al*., 2006; Ask, 2010) indicated that FPD can be reduced by genetic selection independent of body weight achieving this is not easy and requires a long term strategy. Even with genetic selection feed composition together with management practices are recognised to be the most important factors in preventing the occurrence of wet litter - which is believed to be the main risk factor affecting feet quality (EC, 2000).

The positive correlation of body weight with FPD and HB reported in earlier studies (Harms & Simpson, 1975; Hemminga & Vertommen, 1985) was not confirmed in the present investigations were the prevalence was related more to litter quality associated parameters than body weight (see Chapter 3). However it is possible that the body weight may have an indirect effect. More pressure per area of foot, increasing the contact of sensitive areas of the skin to the irritants in the litter (Stephenson *et al*., 1960; Mcllory *et al*., 1987; Menzies *et al*., 1998). It is important to note that most of the studies identifying a link to body weight have not included components associated with litter i.e. NH_3 and pH and therefore failed to highlight the relationship of important contributors towards development and severity of FPD and HB. Similarly studies reporting litter moisture alone can cause FPD (Martland, 1984; Mayne *et al*., 2007) continuously housed the birds on wet litter. The relevance of this to birds housed in commercial units does however require careful interpretation as in the field litter moisture is variable in the poultry house i.e. higher near feeders and drinkers and lower further away from them (Lovanh *et al*., 2007) providing opportunity for the bird to rest in drier conditions.

The previously reported relationship of wet litter conditions and higher volatilization of ammonia from the litter (Elwinger & Svensson 1996; Liu *et al*., 2007; Nahm, 2007) was not supported by the present findings suggesting that it is the presence of the substrate (uric acid) that results in litter ammonia - higher litter moisture may accelerate the release of ammonia by providing suitable conditions for microbial activity but it was not the main reason of litter ammonia ion concentration (Carey *et al*., 2004). Interestingly litter moisture or litter score did not correlate with the prevalence of FPD or HB although these parameters did relate to litter NH_3 and pH concentration (see Chapter 3). Higher NH_3 in the litter is believed to be irritant and causes chemical burning effect on the skin (Homidan *et al*., 2003). Litter moisture cause softening of the skin and make it prone to damage (Mayne *et al*., 2007). Possibly sticky/wet litter brings this irritant in close contact to the foot

pad of the bird (Jensen *et al*., 1970; Pattison, 1987) since there are reports that the contact of the turkeys' feet with the excreta induces FPD (Jensen, 1985; Tucker & Walker, 1992). Therefore high dietary protein level has been found to increase the incidence and severity of HB and FPD in broilers (Bray & Lynn, 1986; Nagaraj *et al*., 2007) due to increased nitrogen excretion by the bird and therefore NH_3 formation in the litter, whereas the presence of wet capped litter appeared to exacerbate the problem. However it is important to establish the threshold levels of both the litter moisture and NH_3 beyond which the damage to the skin starts. These findings also highlight the shortcoming in the current litter scoring, which is based on physical characteristics (only reflecting moisture content) and fails to incorporate important litter chemical properties e.g. NH_3, pH and litter temperature. Since litter NH_3 and pH are closely associated, it would be economically sensible to have a hand held pH meter (see Figure 11 in Chapter 3) to measure litter pH which then can be related to possible NH_3 ion concentration in the litter. This would help the producer to better manage the litter and so control the prevalence of FPD and HB.

Skin exposure time to these irritants is also important. This was also evident in the present findings when evaluating the effect of age. Even though there was a linear effect of age on the increase in litter moisture however there was decline in litter NH_3 as well as FPD and HB prevalence. Previous investigations suggested that the frequency and severity of lesions on the foot-pads, hocks and breast increase with the age of the birds (Greene *et al*., 1985; Hemminga & Vertommen, 1985; Martland, 1985; McIlroy *et al*., 1987). However, the present findings (see Chapters 3 and 4) were more in agreement with the findings of Ekstrand *et al*. (1997), who observed healing of the pododermatitis lesions at an older age provided that birds were fed on less nutrient intense diets. It is important to mention that although the incidences of skin damage reduce as age progressed, the severity increased. Berg (1998) concluded similarly as there was no association between age at slaughter and foot-pad dermatitis, either in broilers nor in turkeys. HB lesions appears first which may have some effect on birds' behaviour and they prefer to stand rather than sit due to pain which means exposing their feet to the factors associated with litter. Once the FPD appears birds had a change in behaviour and they were sitting rather than standing so HB started to reappear.

Although limited information is available dealing with nutritional intervention in the control of pododermatitis (Bilgilis *et al*., 2005; Bilgilis *et al*., 2006) however, higher incidences of feet damage worldwide, economic and welfare importance will likely seek attention from the industry and researchers. Since feed composition is the major contributor to litter quality which primarily causes this problem, the litter associated factors can be controlled through better understanding of the feed composition and applying proper husbandry practices (EC, 2000). The complexity and interrelationship of the factors involved in the

control of litter and therefore in the control of FPD and HB requires further investigations in the light of present findings.

It was observed in the present investigations, reported in Chapters 3 and 4 (although not measured), that spatial effects on litter wetness existed in the pens which resulted in heterogeneity on the wet patches of litter (wetness observed near drinkers and feeders) (Lovanh *et al*., 2007). It was not recorded or the aim of present studies to investigate any behavioural aspect of turkey production, however the author thinks it is important to report as a guideline for any future investigation. The turkeys spend most of their time away from feeders and drinkers and prefer to locate themselves on drier patches (personal observation). Therefore if economically feasible consideration should be given to the use of a differential litter material in the house i.e. near drinkers and feeders e.g. sugar beet pulp (higher capacity of holding moisture and release quickly) and for dry refuge away from the drinker and feeder (e.g. wood shaving) can help birds to stay clean and minimise incidences of feet skin damage. Along with the above mentioned, in future possible mechanisms should be developed to study the water gradient as well as changes in chemical fractions (NH_3 and pH in particular) at different litter depth and their relationship with the incidences and severity of FPD and HB.

6.2 **Future recommendations**

Although the present studies highlighted that components that influence litter quality and associated leg health can be controlled effectively through nutritional modification, it is important to note that management is also an effective tool in maintaining those controls. Therefore recommendations here cover both nutritional and management approaches.

- Need to further investigate and compare the response of turkeys when fed lower dietary protein (containing higher AME:CP) supplied by various vegetable protein sources supplemented with crystalline amino acids on nutrient digestibility, nitrogen excretion, water utilisation, litter quality and leg health (FPD and HB) parameters.

- Similar to above but formulated with 3-5% fixed fish meal concentration. This may help to improve the amino acid digestibility and balance and may result better protein utilisation and litter quality.

- Gradual decrease in ideal protein concentration in different phases (shorter phases) from the commercial recommendation (achieved by mixing diets with

varying nutrient density) may help to maintain production standards along with litter quality and bird wellbeing.

- Above mentioned approaches but comparing mash feed form verses pelleted diets and evaluating which type of feeding would help effectively control litter quality issues. Also compare the economic advantages and disadvantages of these feeding regimes for turkeys.

- Compare different strains and genders of turkeys under similar environment and evaluate their ability to withstand the conditions against the prevalence of leg health issues.

- Dietary supplementation of enzymes such as carbohydrases in diets containing high quantities of soybean meal (all vegetable protein sources) along with some litter amendments may provide extra benefits.

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Appendix 1

Table 53: Effect of dietary nutrient concentrations on leg health parameters.

	BFPS	BHS	GFPS	GHS	TFPS	THS
Week 8						
T1+8	0	0.457	0	0.543	0	0.543
T2+8	0	0.400	0	0.600	0	0.571
T3+8	0	0.500	0	0.500	0	0.621
T4+8	0	0.686	0	0.314	0	0.800
T5+8	0	0.679	0	0.321	0	1.093
SEM	0	0.1236	0	0.1236	0	0.1743
			Probabilities of statistical differences			
Diet	-	NS	-	NS	-	<0.05
L	-	<0.05	-	<0.05	-	<0.01
Q	-	NS	-	NS	-	NS
Contrast 1	-	NS	-	NS	-	NS
Contrast 2	-	NS	-	NS	-	<0.05
Week 12						
T1+12	0.250	0.243	0.750	0.757	0.350	0.300
T2+12	0.271	0.193	0.729	0.807	0.357	0.371
T3+12	0.336	0.336	0.664	0.664	0.286	0.486
T4+12	0.286	0.229	0.714	0.771	0.479	0.286
T5+12	0.250	0.521	0.750	0.479	0.279	1.064
SEM	0.1535	0.1190	0.1535	0.1190	0.2441	0.1868
			Probabilities of statistical differences			
Diet	NS	P=0.07	NS	P=0.07	NS	<0.01
L	NS	<0.05	NS	<0.05	NS	<0.01
Q	NS	NS	NS	NS	NS	<0.05
Contrast 1	NS	NS	NS	NS	NS	NS
Contrast 2	NS	NS	NS	NS	NS	NS
Week 16						
T1+16	0.000	0.221	1.000	0.779	0.000	0.250
T2+16	0.029	0.064	0.971	0.936	0.029	0.150
T3+16	0.029	0.186	0.971	0.814	0.029	0.314
T4+16	0.057	0.200	0.943	0.800	0.086	0.371
T5+16	0.036	0.271	0.964	0.729	0.036	0.579
SEM	0.0524	0.1036	0.0524	0.1036	0.0668	0.2111
			Probabilities of statistical differences			
Diet	NS	NS	NS	NS	NS	NS
L	NS	NS	NS	NS	NS	P=0.07
Q	NS	NS	NS	NS	NS	NS
Contrast 1	NS	NS	NS	NS	NS	NS
Contrast 2	NS	NS	NS	NS	NS	NS
Week 20						
T1+20	0.121	0.193	0.879	0.807	0.150	0.221
T2+20	0.064	0.029	0.936	0.971	0.093	0.114
T3+20	0.036	0.350	0.964	0.650	0.036	0.543
T4+20	0.086	0.207	0.914	0.793	0.114	0.393
T5+20	0.000	0.293	1.000	0.707	0.000	0.736
SEM	0.0663	0.1179	0.0663	0.1179	0.0918	0.2210
			Probabilities of statistical differences			
Diet	NS	NS	NS	NS	NS	P=0.07
L	NS	NS	NS	NS	NS	<0.05
Q	NS	NS	NS	NS	NS	NS
Contrast 1	NS	NS	NS	<0.05	NS	P=0.07
Contrast 2	NS	NS	NS	NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 54: Effect of treatments on total weight gain ((TWG)kg/b/4 weeks), weight gain ((WG)kg/b/d), crude fat intake ((C.FI) g/b/d), crude protein intake ((CPI) g/b/d), feed intake ((FI) kg/b/d), water intake ((WI) kg/b/d), feed intake for water ratio feed (FI W:F) kg/b/d), water ratio feed ((W:F) kg/kg), Litter NH_3 ((NH_3) ppm).

	TWG	WG	C. F I	CPI	FI	WI	FI W:F	W:F	NH_3
Week 8									
T1+8	3.18	0.114	6.28	40.11	0.208	0.439	0.227	1.93	2.89
T2+8	3.25	0.116	9.88	45.39	0.211	0.459	0.222	2.07	3.39
T3+8	3.32	0.119	15.62	51.70	0.201	0.452	0.209	2.15	3.38
T4+8	3.41	0.122	19.04	55.30	0.194	0.501	0.224	2.24	2.63
T5+8	3.53	0.126	22.70	59.86	0.192	0.506	0.214	2.36	4.01
SEM	0.046	0.0017	0.367	0.974	0.0041	0.0247	0.0057	0.096	0.303
Probabilities of statistical differences									
Diet	<0.001	<0.001	<0.001	<0.001	<0.05	NS	NS	<0.05	NS
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	NS	<0.01	NS
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	P=0.06	P=0.06	<0.001	<0.001	NS	NS	<0.05	NS	NS
Contrast 2	<0.05	<0.05	<0.001	<0.001	NS	NS	NS	NS	NS
Week 12									
T1+12	4.62	0.165	10.63	69.7	0.446	0.792	0.471	1.69	12.50
T2+12	4.92	0.176	20.82	80.7	0.456	0.841	0.478	1.77	13.14
T3+12	5.09	0.182	36.32	91.0	0.425	0.858	0.459	1.86	14.84
T4+12	5.10	0.182	46.90	100.3	0.420	0.736	0.432	1.71	15.07
T5+12	5.26	1.88	54.45	104.1	0.396	0.710	0.402	1.77	16.54
SEM	0.103	0.0037	1.113	2.33	0.0111	0.0442	0.0179	0.058	0.892
Probabilities of statistical differences									
Diet	<0.01	<0.01	<0.001	<0.001	<0.01	NS	<0.05	NS	<0.05
L	<0.001	<0.001	<0.001	<0.001	<0.001	P=0.07	<0.01	NS	<0.01
Q	NS	NS	<0.05	NS	NS	P=0.09	NS	NS	NS
Contrast 1	<0.05	<0.05	<0.001	<0.001	P=0.07	NS	NS	P=0.08	P=0.08
Contrast 2	NS	NS	<0.001	<0.001	NS	<0.05	P=0.07	NS	NS
Week 16									
T1+16	5.02	0.179	12.7	87.3	0.632	1.004	0.640	1.58	7.07
T2+16	5.12	0.183	27.1	104.0	0.663	0.922	0.629	1.48	7.07
T3+16	5.09	0.182	46.1	111.4	0.583	0.832	0.581	1.44	10.81
T4+16	5.20	0.186	60.7	124.3	0.582	0.767	0.551	1.40	10.79
T5+16	5.30	0.189	70.1	128.0	0.541	0.751	0.505	1.50	12.71
SEM	0.130	0.0046	2.42	5.10	0.0258	0.0513	0.0340	0.058	0.660
Probabilities of statistical differences									
Diet	NS	NS	<0.001	<0.001	<0.05	<0.01	P=0.05	NS	<0.001
L	NS	NS	<0.001	<0.001	<0.01	<0.001	<0.01	NS	<0.001
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	NS	NS	<0.001	<0.05	P=0.05	<0.05	NS	NS	<0.001
Contrast 2	NS	NS	<0.001	<0.05	NS	NS	NS	NS	NS
Week 20									
T1+20	3.65	0.130	12.7	75.8	0.632	1.136	0.660	1.73	3.79
T2+20	4.52	0.161	33.5	100.0	0.747	1.070	0.742	1.45	3.86
T3+20	4.75	0.170	57.8	101.9	0.640	1.023	0.665	1.53	4.71
T4+20	4.24	0.152	64.4	94.1	0.534	0.946	0.624	1.52	7.00
T5+20	4.55	0.163	77.3	98.9	0.512	0.768	0.486	1.61	5.14
SEM	0.178	0.0063	2.73	4.47	0.028	0.0731	0.0378	0.077	0.375
Probabilities of statistical differences									
Diet	<0.01	<0.01	<0.001	<0.01	<0.001	<0.05	<0.001	NS	<0.001
L	<0.05	<0.05	<0.001	<0.05	<0.001	<0.001	<0.001	NS	<0.001
Q	<0.05	<0.05	<0.01	<0.05	<0.05	NS	<0.01	P=0.05	P=0.09
Contrast 1	<0.01	<0.01	<0.001	<0.05	NS	NS	NS	NS	P=0.06
Contrast 2	NS	NS	<0.001	NS	<0.01	P=0.08	<0.05	NS	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 55: Effect of treatments on intake (units g/b/d until specicified) of ash (AshI), calcium (CaI), Copper (CuI) mg/b/d), potassium (KI), magnesium (MgI), manganes ((MnI) mg/b/d), sodium (NaI), phosphorus (PI), sulphur ((SI) mg/b/d) and zinc ((Zn) mg/b/d).

	Ash I	Ca I	Cu I	K I	Mg I	Mn I	Na I	P I	S I	Zn I
Week 8										
T1+8	13.44	2.42	3.88	1.99	0.42	28.87	0.25	1.79	654.9	25.98
T2+8	13.66	2.40	4.14	2.08	0.42	28.46	0.27	1.83	717.7	26.99
T3+8	13.12	2.18	4.01	2.11	0.38	25.79	0.30	1.76	780.5	26.96
T4+8	12.72	2.04	3.91	2.12	0.36	24.04	0.32	1.71	814.9	26.80
T5+8	12.62	1.95	3.89	2.17	0.35	22.86	0.35	1.70	864.5	27.20
SEM	0.266	0.047	0.079	0.041	0.008	0.559	0.012	0.036	14.70	0.527
	Probabilities of statistical differences									
Diet	<0.05	<0.001	NS	<0.05	<0.001	<0.001	<0.001	NS	<0.001	NS
L	<0.01	<0.001	NS	<0.01	<0.001	<0.001	<0.001	<0.05	<0.001	NS
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.05	<0.001	NS	NS	<0.01	<0.001	<0.01	NS	<0.001	NS
Contrast 2	NS	<0.01	NS	NS	<0.01	<0.01	<0.05	NS	<0.01	NS
Week 12										
T1+12	26.56	4.29	7.17	3.45	0.87	53.89	0.27	3.57	1264	55.40
T2+12	26.95	4.34	7.52	3.65	0.85	54.17	0.35	3.61	1408	58.59
T3+12	24.69	3.92	7.34	3.60	0.76	48.78	0.43	3.28	1506	57.81
T4+12	24.16	3.84	7.46	3.66	0.75	47.18	0.49	3.16	1615	59.26
T5+12	22.52	3.54	7.22	3.57	0.67	43.43	0.54	2.94	1640	57.79
SEM	0.651	0.104	0.189	0.092	0.021	1.298	0.017	0.087	38.4	1.484
	Probabilities of statistical differences									
Diet	<0.001	<0.001	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS
L	<0.001	<0.001	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.05	<0.01	NS	NS	<0.001	<0.01	<0.001	<0.01	<0.001	NS
Contrast 2	NS	P=0.08	NS	NS	P=0.07	<0.05	<0.001	<0.05	<0.05	Ns
Week 16										
T1+16	32.52	5.47	11.43	4.29	1.07	78.9	0.43	4.67	1819	72.4
T2+16	34.40	5.81	12.92	4.62	1.07	84.0	0.51	4.91	2037	77.4
T3+16	30.65	5.21	12.84	4.17	0.89	75.6	0.55	4.31	1994	70.2
T4+16	30.86	5.25	13.82	4.26	0.84	76.6	0.58	4.36	2126	71.4
T5+16	28.97	4.94	13.79	4.06	0.74	72.4	0.60	4.06	2103	67.8
SEM	1.360	0.231	0.579	0.186	0.039	3.36	0.028	0.193	89.6	3.12
	Probabilities of statistical differences									
Diet	P=0.09	NS	<0.05	NS	<0.001	NS	<0.01	<0.05	NS	NS
L	<0.05	<0.05	<0.01	NS	<0.001	<0.05	<0.001	<0.01	<0.05	NS
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	NS	NS	NS	NS	<0.001	NS	<0.05	P=0.06	NS	NS
Contrast 2	NS	NS	NS	NS	<0.05	NS	NS	NS	NS	NS
Week 20										
T1+20	29.33	5.37	11.17	3.81	1.02	77.9	0.48	4.24	1590	77.3
T2+20	34.28	6.30	12.91	4.52	1.15	91.1	0.64	5.09	2016	93.3
T3+20	28.68	5.24	10.60	3.88	0.87	76.5	0.60	4.42	1941	82.8
T4+20	23.64	4.34	8.60	3.25	0.69	63.1	0.55	3.75	1741	70.8
T5+20	22.31	4.08	7.99	3.12	0.62	59.7	0.57	3.65	1783	69.4
SEM	1.279	0.234	0.474	0.172	0.040	3.41	0.028	0.196	85.2	3.66
	Probabilities of statistical differences									
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.05	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	NS	<0.01
Q	<0.05	<0.05	<0.05	<0.05	P=0.09	<0.05	<0.05	<0.05	<0.05	<0.05
Contrast 1	P=0.06	P=0.05	<0.05	NS	<0.001	P=0.07	NS	NS	NS	NS
Contrast 2	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	NS	<0.01	NS	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 56: Effect of treatments on intake (g/b/d) of alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), histidine (His), isoleucine (Ile) and leucine (Leu).

	Ala	Arg	Asp	Glu	His	Ile	Leu
Week 8							
T1+8	1.44	2.04	3.50	8.30	0.74	1.73	2.82
T2+8	1.67	2.32	4.04	9.17	0.85	2.00	3.45
T3+8	1.96	2.65	4.73	10.08	0.96	2.34	3.79
T4+8	2.12	2.83	5.12	10.59	1.06	2.54	4.10
T5+8	2.32	3.07	5.61	11.29	1.16	2.78	4.47
SEM	0.037	0.050	0.090	0.190	0.019	0.044	0.072
	Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	P=0.06	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001
Week 12							
T1+12	2.31	3.00	5.14	13.71	1.15	2.66	4.60
T2+12	2.77	3.62	6.43	15.81	1.41	3.28	5.63
T3+12	3.27	4.30	7.97	17.75	1.71	4.00	6.81
T4+12	3.67	4.85	9.17	19.52	1.95	4.57	7.76
T5+12	3.88	5.13	9.85	20.23	2.08	4.88	8.26
SEM	0.0844	0.111	0.209	0.454	0.045	0.105	0.177
	Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	P=0.09	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 16							
T1+16	2.98	3.73	6.09	20.36	1.53	3.36	5.82
T2+16	3.67	4.59	7.71	23.50	1.89	4.16	7.07
T3+16	4.11	5.12	8.90	24.09	2.12	4.69	7.78
T4+16	4.68	5.83	10.28	26.31	2.42	5.36	8.79
T5+16	4.89	6.09	10.87	26.59	2.54	5.62	9.14
SEM	0.190	0.237	0.416	1.092	0.098	0.218	0.359
	Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.01	<0.01	<0.001	NS	<0.01	<0.01	<0.01
Contrast 2	<0.01	<0.01	<0.01	P=0.09	<0.01	<0.01	<0.05
Week 20							
T1+20	2.36	2.94	4.64	18.57	1.29	2.72	4.90
T2+20	3.21	3.98	6.67	23.67	1.70	3.81	6.69
T3+20	3.41	4.21	7.59	22.99	1.73	4.21	7.13
T4+20	3.21	3.96	7.38	20.70	1.60	4.04	6.73
T5+20	3.43	4.22	8.07	21.29	1.68	4.38	7.20
SEM	0.150	0.185	0.336	1.009	0.076	0.186	0.314
	Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.05	<0.01	<0.001	<0.001
L	<0.001	<0.001	<0.001	NS	<0.05	<0.001	<0.001
Q	<0.05	<0.05	<0.01	<0.05	<0.05	<0.01	<0.05
Contrast 1	<0.01	<0.01	<0.001	NS	<0.05	<0.001	<0.01
Contrast 2	NS	NS	NS	NS	NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 57: Effect of treatments on intake (g/b/d) of lysine (Lys), phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val).

	Lys	Phe	Ser	Thr	Tyr	Val
Week 8						
T1+8	2.21	1.87	1.25	1.46	1.04	1.83
T2+8	2.54	2.11	1.45	1.72	1.21	2.09
T3+8	2.96	2.41	1.71	2.08	1.41	2.42
T4+8	3.20	2.58	1.86	2.28	1.53	2.60
T5+8	3.49	2.79	2.04	2.53	1.68	2.83
SEM	0.056	0.045	0.032	0.040	0.027	0.046
	Probabilities of statistical differences					
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 12						
T1+12	3.84	2.95	1.95	2.13	1.58	3.05
T2+12	4.46	3.58	2.38	2.71	1.94	3.60
T3+12	5.07	4.29	2.87	3.43	2.37	4.17
T4+12	5.60	4.87	3.26	3.97	2.71	4.66
T5+12	5.83	5.17	3.46	4.29	2.89	4.89
SEM	0.130	0.112	0.075	0.091	0.062	0.107
	Probabilities of statistical differences					
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	P=0.08	P=0.09	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 16						
T1+16	5.41	3.89	2.52	2.88	1.83	4.20
T2+16	6.42	4.65	3.14	3.71	2.26	5.06
T3+16	6.84	5.00	3.56	4.35	2.54	5.48
T4+16	7.61	5.59	4.07	5.07	2.90	6.16
T5+16	7.81	5.77	4.28	4.93	3.04	6.37
SEM	0.313	0.229	0.165	0.196	0.118	0.252
	Probabilities of statistical differences					
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	<0.05	NS	NS
Contrast 1	<0.05	<0.05	<0.001	<0.001	<0.01	<0.05
Contrast 2	<0.05	<0.05	<0.01	<0.05	<0.01	<0.05
Week 20						
T1+20	3.77	3.34	2.03	1.61	1.32	3.23
T2+20	4.93	4.47	2.74	2.33	1.86	4.41
T3+20	4.97	4.64	2.88	2.68	2.09	4.72
T4+20	4.57	4.34	2.70	2.62	2.02	4.46
T5+20	4.78	4.59	2.88	2.87	2.19	4.77
SEM	0.218	0.204	0.127	0.119	0.092	0.208
	Probabilities of statistical differences					
Diet	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.05	<0.01	<0.001	<0.001	<0.001	<0.001
Q	<0.05	<0.05	<0.05	<0.01	<0.05	<0.05
Contrast 1	<0.05	<0.01	<0.01	<0.001	<0.001	<0.01
Contrast 2	NS	NS	NS	NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low nutrient concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high nutrient concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Appendix 2

Table 58: Effect of dietary protein concentrations on leg health parameters.

	BFPS	BHS	GFPS	GHS	TFPS	THS
Week 8						
T1+8	0	0.343	0	0.657	0	0.429
T2+8	0	0.457	0	0.543	0	0.514
T3+8	0	0.414	0	0.586	0	0.586
T4+8	0	0.571	0	0.429	0	0.771
T5+8	0	0.464	0	0.536	0	0.664
SEM	0	0.1057	0	0.1057	0	0.1773
	Probabilities of statistical differences					
Diet	-	NS	-	NS	-	NS
L	-	NS	-	NS	-	NS
Q	-	NS	-	NS	-	NS
Contrast 1	-	NS	-	NS	-	NS
Contrast 2	-	NS	-	NS	-	NS
Week 12						
T1+12	0.029	0.057	0.971	0.943	0.029	0.171
T2+12	0.093	0.057	0.907	0.943	0.129	0.057
T3+12	0.114	0.257	0.886	0.743	0.143	0.600
T4+12	0.286	0.500	0.714	0.500	0.314	0.900
T5+12	0.357	0.486	0.643	0.514	0.471	0.771
SEM	0.0638	0.0828	0.0638	0.0828	0.0971	0.1819
	Probabilities of statistical differences					
Diet	<0.01	<0.001	<0.01	<0.001	<0.05	<0.05
L	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001
Q	NS	NS	NS	NS	NS	NS
Contrast 1	NS	P=0.06	NS	P=0.06	NS	<0.05
Contrast 2	<0.05	<0.05	<0.05	<0.05	<0.05	NS
Week 16						
T1+16	0.036	0.029	0.964	0.971	0.036	0.114
T2+16	0.029	0.057	0.971	0.943	0.029	0.057
T3+16	0.086	0.200	0.914	0.800	0.114	0.400
T4+16	0.207	0.286	0.793	0.714	0.207	0.486
T5+16	0.157	0.250	0.843	0.750	0.157	0.436
SEM	0.0443	0.0598	0.0443	0.0598	0.0505	0.1341
	Probabilities of statistical differences					
Diet	<0.05	<0.05	<0.05	<0.05	P=0.08	NS
L	<0.01	<0.001	<0.01	<0.001	<0.05	<0.05
Q	NS	NS	NS	NS	NS	NS
Contrast 1	NS	<0.05	NS	<0.05	NS	P=0.07
Contrast 2	P=0.09	NS	P=0.09	NS	NS	NS
Week 20						
T1+20	0.129	0.229	0.871	0.771	0.157	0.364
T2+20	0.093	0.157	0.907	0.843	0.157	0.350
T3+20	0.143	0.400	0.857	0.600	0.214	0.771
T4+20	0.129	0.121	0.871	0.879	0.164	0.179
T5+20	0.000	0.086	1.000	0.914	0.000	0.086
SEM	0.0615	0.0894	0.0615	0.0894	0.0840	0.1533
	Probabilities of statistical differences					
Diet	NS	NS	NS	NS	NS	<0.05
L	NS	NS	NS	NS	NS	NS
Q	NS	NS	NS	NS	NS	<0.05
Contrast 1	P=0.07	NS	NS	P=0.07	NS	<0.05
Contrast 2	NS	P=0.09	NS	<0.05	NS	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 59: Effect of treatments on total weight gain ((TWG)kg/b/4 weeks), weight gain ((WG)kg/b/d), feed efficiency ((FE) kg WG/kg FI), litter pH, litter moisture ((LM) g/kg), Litter NH_3 ((NH_3) ppm) and litter score (LS).

	TWG	WG	FE	W:F	pH	LM	NH_3	LS
Week 8								
T1+8	2.90	0.104	0.532	1.74	7.22	222.7	3.08	1.20
T2+8	3.09	0.110	0.566	1.95	7.20	213.1	2.66	1.33
T3+8	3.37	0.120	0.601	2.31	7.56	247.3	2.86	1.43
T4+8	3.56	0.127	0.598	2.42	8.38	275.1	3.79	1.56
T5+8	3.52	0.126	0.610	2.27	7.94	243.1	3.26	1.56
SEM	0.050	0.0018	0.0085	0.064	0.167	14.33	0.291	0.071
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	NS	<0.01
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	NS	<0.001
Q	<0.01	<0.01	<0.05	<0.001	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	NS	NS	NS	P=0.07
Contrast 2	<0.05	<0.05	NS	NS	<0.01	NS	P=0.08	NS
Week 12								
T1+12	3.93	0.141	0.406	1.79	8.49	292.1	13.07	1.61
T2+12	4.66	0.166	0.422	1.84	8.50	295.0	15.14	1.51
T3+12	5.03	0.180	0.432	2.06	8.63	316.1	16.54	1.89
T4+12	5.32	0.190	0.433	2.18	8.76	344.6	16.21	2.14
T5+12	5.18	0.185	0.453	2.23	8.87	354.9	20.36	2.26
SEM	0.094	0.0034	0.0088	0.104	0.105	20.56	1.103	0.135
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.05	<0.05	P=0.09	NS	<0.01	<0.01
L	<0.001	<0.001	<0.001	<0.001	<0.01	<0.05	<0.001	<0.001
Q	<0.001	<0.001	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	NS	P=0.08	NS	NS	P=0.08	P=0.06
Contrast 2	P=0.07	P=0.07	NS	NS	NS	NS	NS	P=0.07
Week 16								
T1+16	4.92	0.176	0.363	1.40	7.95	251.9	7.07	1.29
T2+16	4.95	0.177	0.311	1.47	8.15	277.4	7.36	1.57
T3+16	4.95	0.177	0.322	1.49	8.28	358.8	10.50	1.99
T4+16	5.31	0.90	0.314	1.59	8.49	392.1	13.36	2.19
T5+16	5.14	0.184	0.320	1.70	8.52	425.8	16.79	2.39
SEM	0.122	0.0044	0.0147	0.059	0.067	19.05	0.516	0.093
	Probabilities of statistical differences							
Diet	NS	NS	NS	<0.05	<0.01	<0.001	<0.001	<0.001
L	P=0.05	P=0.05	NS	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	<0.01	NS
Contrast 1	NS	NS	NS	NS	<0.01	<0.001	<0.001	<0.001
Contrast 2	P=0.08	P=0.08	NS	P=0.05	<0.05	<0.05	<0.001	<0.05
Week 20								
T1+20	4.28	0.153	0.273	1.39	7.57	235.0	2.50	1.34
T2+20	3.82	0.136	0.229	1.30	7.68	292.0	2.50	1.60
T3+20	4.08	0.146	0.231	1.46	7.89	355.0	4.71	1.62
T4+20	4.26	0.152	0.226	1.44	8.37	389.0	7.79	2.09
T5+20	4.68	0.167	0.287	1.60	8.36	404.0	12.86	2.04
SEM	0.223	0.0080	0.0127	0.055	0.101	25.3	0.615	0.089
	Probabilities of statistical differences							
Diet	NS	NS	<0.01	<0.05	<0.001	<0.001	<0.001	<0.001
L	P=0.09	P=0.09	NS	<0.01	<0.001	<0.001	<0.001	<0.001
Q	P=0.07	P=0.07	<0.001	NS	NS	NS	<0.001	NS
Contrast 1	NS	NS	<0.001	P=0.09	<0.05	<0.01	<0.01	NS
Contrast 2	NS	NS	P=0.05	NS	<0.001	NS	<0.001	<0.001

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 60: Effect of treatments on intake (units g/b/d until specicified) of ash (AshI), crude fat (C.FI), crude protein (CPI), Copper (CuI) mg/b/d), potassium (KI), magnesium (MgI), manganes ((MnI) mg/b/d), sodium (NaI), sulphur ((SI) mg/b/d) and zinc ((Zn) mg/b/d).

	Ash I	C. F I	CPI	Cu I	K I	Mg I	Mn I	Na I	S I	Zn I
Week 8										
T1+8	12.68	7.21	35.29	3.68	1.32	0.25	21.51	0.31	561	36.07
T2+8	12.81	7.49	39.13	3.73	1.49	0.27	23.33	0.30	611	33.78
T3+8	13.52	8.28	47.83	3.96	1.87	0.33	27.62	0.32	730	30.66
T4+8	14.52	9.14	55.77	4.27	2.21	0.37	31.68	0.34	841	29.50
T5+8	14.30	9.25	59.17	4.23	2.37	0.39	33.15	0.34	883	25.80
SEM	0.276	0.173	1.093	0.081	0.044	0.007	0.611	0.014	16.3	0.673
					Probabilities of statistical differences					
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.05	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	NS	<0.001	<0.001
Contrast 2	<0.05	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	NS	<0.001	<0.001
Week 12										
T1+12	17.64	15.04	52.82	6.49	2.01	0.41	38.05	0.32	950	45.42
T2+12	20.56	17.43	68.08	7.07	2.51	0.50	46.76	0.40	1174	50.90
T3+12	22.79	19.12	87.78	6.90	3.15	0.60	56.36	0.50	1427	53.09
T4+12	24.78	20.64	103.52	6.87	3.66	0.71	64.23	0.55	1633	55.23
T5+12	23.67	19.59	106.14	5.99	3.70	0.69	64.02	0.55	1633	50.53
SEM	0.517	0.434	2.058	0.167	0.073	0.014	1.285	0.022	32.6	1.230
					Probabilities of statistical differences					
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001
Contrast 1	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Contrast 2	<0.05	P=0.07	<0.001	<0.05	<0.001	<0.001	<0.001	<0.05	<0.001	NS
Week 16										
T1+16	24.21	35.91	72.76	14.69	3.03	0.73	59.78	0.38	1418	53.37
T2+16	27.83	38.72	90.83	15.51	3.58	0.82	68.75	0.47	1722	62.71
T3+16	27.88	34.27	103.45	13.12	3.81	0.80	68.89	0.53	1889	65.13
T4+16	31.28	35.26	124.81	13.00	4.40	0.88	77.27	0.62	2234	74.73
T5+16	30.12	31.00	128.30	10.92	4.36	0.83	74.44	0.64	2258	73.46
SEM	1.016	1.427	3.373	0.577	0.132	0.030	2.509	0.023	63.4	2.292
					Probabilities of statistical differences					
Diet	<0.001	<0.05	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.01	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	NS	P=0.09	<0.001	<0.01	<0.01	NS	NS	<0.01	<0.001	<0.05
Contrast 2	<0.05	NS	<0.001	NS	<0.01	NS	<0.05	<0.001	<0.001	<0.01
Week 20										
T1+20	24.36	42.93	67.63	8.88	2.71	0.62	69.34	0.48	1534	60.79
T2+20	25.88	43.10	79.53	9.62	3.03	0.67	73.38	0.52	1673	65.13
T3+20	28.21	41.86	101.81	10.85	3.62	0.76	79.48	0.60	1911	72.11
T4+20	30.67	41.97	121.38	12.07	4.16	0.84	86.13	0.65	2140	79.56
T5+20	26.79	32.95	112.89	10.55	3.75	0.74	73.49	0.59	1884	68.56
SEM	1.363	2.038	4.933	0.524	0.175	0.037	3.843	0.029	92.3	3.485
					Probabilities of statistical differences					
Diet	<0.05	<0.01	<0.001	<0.01	<0.001	<0.01	<0.05	<0.01	<0.001	<0.05
L	<0.05	<0.01	<0.001	<0.001	<0.001	<0.001	P=0.08	<0.001	<0.001	<0.01
Q	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Contrast 1	P=0.08	<0.001	<0.05	NS	<0.01	<0.05	NS	<0.01	<0.05	<0.05
Contrast 2	NS	<0.05	NS	NS	NS	NS	NS	NS	NS	NS

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 61: Effect of treatments on intake (g/b/d) of alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), histidine (His), isoleucine (Ile) and leucine (Leu).

	Ala	Arg	Asp	Glu	Gly	His	Ile	Leu
Week 8								
T1+8	1.11	1.36	2.50	7.10	1.07	0.51	1.39	2.32
T2+8	1.32	1.65	3.00	7.82	1.25	0.62	1.60	2.67
T3+8	1.77	2.26	4.06	9.46	1.64	0.86	2.07	3.43
T4+8	2.14	2.78	4.96	10.98	1.98	1.06	2.48	4.10
T5+8	2.35	3.08	5.47	11.60	2.16	1.18	2.69	4.44
SEM	0.045	0.059	0.104	0.214	0.041	0.023	0.050	0.083
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 12								
T1+12	1.81	2.18	4.08	11.59	1.69	0.91	2.26	3.78
T2+12	2.31	2.97	5.34	14.42	2.39	1.28	2.91	4.95
T3+12	2.93	4.10	7.02	17.71	3.43	1.82	3.75	6.51
T4+12	3.42	4.98	8.35	20.39	4.24	2.25	4.42	7.74
T5+12	3.48	5.23	8.62	20.49	4.51	2.39	4.54	7.99
SEM	0.068	0.100	0.166	0.406	0.086	0.046	0.088	0.154
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	<0.001	<0.01	<0.01	<0.001	<0.01	<0.01	<0.001	<0.01
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 16								
T1+16	2.57	3.41	6.17	18.38	2.37	1.35	3.28	5.56
T2+16	3.23	4.20	7.65	22.05	3.14	1.69	4.05	6.86
T3+16	3.73	4.70	8.64	23.73	3.87	1.94	4.55	7.69
T4+16	4.53	5.62	10.38	27.77	4.86	2.36	5.45	9.21
T5+16	4.68	5.73	10.64	27.81	5.16	2.43	5.57	9.41
SEM	0.121	0.155	0.284	0.810	0.120	0.063	0.150	0.254
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 20								
T1+20	1.91	2.41	4.81	17.21	2.07	1.10	2.75	4.85
T2+20	2.37	2.92	5.92	19.72	2.54	1.32	3.34	5.90
T3+20	3.26	3.90	8.04	24.33	3.46	1.74	4.46	7.90
T4+20	4.02	4.74	9.87	28.46	4.23	2.10	5.44	9.62
T5+20	3.84	4.48	9.40	26.05	4.03	1.97	5.14	9.11
SEM	0.159	0.189	0.391	1.177	0.168	0.084	0.217	0.384
	Probabilities of statistical differences							
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.

Table 62: Effect of treatments on intake (g/b/d) of lysine (Lys), phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val).

	Lys	Phe	Ser	Thr	Tyr	Val
Week 8						
T1+8	1.62	1.50	1.01	0.98	0.74	1.61
T2+8	1.91	1.70	1.19	1.25	0.87	1.80
T3+8	2.55	2.16	1.58	1.80	1.14	2.24
T4+8	3.09	2.56	1.90	2.26	1.38	2.63
T5+8	3.39	2.76	2.08	2.54	1.50	2.81
SEM	0.064	0.051	0.039	0.050	0.028	0.052
Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 12						
T1+12	3.18	2.41	1.67	1.80	1.23	2.40
T2+12	3.92	3.16	2.15	2.47	1.67	3.08
T3+12	4.76	4.17	2.76	3.44	2.31	3.94
T4+12	5.45	4.98	3.25	4.19	2.81	4.63
T5+12	5.45	5.15	3.33	4.42	2.95	4.74
SEM	0.109	0.099	0.065	0.084	0.056	0.092
Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	<0.001	<0.01	<0.001	<0.01	<0.01	<0.001
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 16						
T1+16	4.62	3.70	2.26	2.69	2.08	3.47
T2+16	5.64	4.50	2.84	3.43	2.53	4.27
T3+16	6.24	4.94	3.27	4.03	2.77	4.77
T4+16	7.41	5.84	3.96	4.95	3.28	5.70
T5+16	7.51	5.90	4.09	5.15	3.31	5.81
SEM	0.208	0.166	0.106	0.129	0.093	0.158
Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	NS	NS	NS	NS	NS	NS
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Week 20						
T1+20	4.04	3.35	1.39	1.52	1.38	3.07
T2+20	4.68	3.97	1.83	1.92	1.74	3.67
T3+20	5.87	5.13	2.68	2.68	2.43	4.82
T4+20	6.92	6.14	3.40	3.32	30.03	5.82
T5+20	6.38	5.73	3.31	3.19	2.91	5.46
SEM	0.284	0.248	0.131	0.130	0.119	0.234
Probabilities of statistical differences						
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Q	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Contrast 1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Contrast 2	<0.05	<0.05	<0.001	<0.001	<0.001	<0.01

There is a statistical significant difference when P<0.05; SEM- pooled standard errors of mean; Contrast 1 – Comparison between control (T3) and low dietary CP concentration (T1 and T2, 77 and 85% of standard breed recommendation, respectively) diets. Contrast 2 – Comparison between control (T3) and high dietary CP concentration (T4 and T5, 110 and 120% of standard breed recommendation, respectively) diets. There were 7 observations per treatment.